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ADRENAL SECRETION IN MAN

THE REACTIONS OF THE BLOOD VESSELS OF THE HUMAN EXTREMITY, SENSITIZED BY SYMPATHECTOMY, TO ADRENALIN AND TO ADRENAL SECRETION RESULTING FROM INSULIN HYPOGLYCEMIA

NORMAN E. FREEMAN, REGINALD H. SMITHWICK AND JAMES C. WHITE

WITH THE COLLABORATION OF BRADFORD CANNON AND HENRY HEYL

From the Surgical Laboratory of the Massachusetts General Hospital and the Harvard Medical School

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In the course of observations to be published (1933) on the surface temperature in cases of Raynaud's disease, definite evidence was obtained of the occurrence of vasoconstriction in completely sympathectomized regions upon appropriate stimulation. The stimuli which precipitated the attacks in our cases were cold, pain, and fear. Since it is recognized that increased secretion from the adrenal medulla takes place in animals (Cannon, 1919; Cannon, Querido, Britton and Bright, 1927) under these circumstances, it was possible that the vasospasm observed in the denervated human extremities was also due to an increase in adrenal secretion. Up to the present, however, active medulli-adrenal secretion has not been demonstrated in the intact human body.

Structures which receive their nerve supply from the sympathetic system become more sensitive to adrenalin after denervation, as Elliott (1905) first demonstrated in animals. It seemed possible that sensitization of the blood vessels to adrenalin would also occur after sympathectomy in man. The adrenin secreted under the influence of cold, pain, or fear, acting upon sensitized structures, might thus produce the vasoconstriction noted in our cases. Experiments were therefore performed to demonstrate the sensitization of human blood vessels to injected adrenalin and the time interval between sympathectomy and the onset of sensitization. The reactions of the blood vessels to adrenin secreted by the patient upon appropriate stimulation were also studied. Results obtained on man were confirmed

in cats and rabbits on which control experiments, after exclusion of the adrenals, could be performed. In order to present a complete picture of our findings which bear on the important question of human medulli-adrenal activity, it will be necessary to restate some of our clinical observations which are being reported elsewhere (1934) and to add certain animal experiments which compare the reactions of denervated arteries to insulin hypoglycemia in the presence and in the absence of an active adrenal medulla.

METHODS. The subjects were patients from the Peripheral Vascular, the Neurosurgical, and the Medical Services of the Massachusetts General Hospital. They had all recovered from paravertebral alcohol injections (White, 1930) or operations at which parts of the sympathetic nervous system had been removed because of Raynaud's disease, epilepsy, or bronchial asthma. The results obtained in cases of peripheral vascular disease were therefore corroborated in cases which presented a normal blood supply to the extremities. The completeness of the sympathectomy was confirmed in each case by appropriate tests (White, 1932). Evidence of vasoconstriction was obtained by determinations of the surface temperature of the terminal phalanges with a thermocouple (Dermatherm, Taylor Instrument Co., Rochester, N. Y.), by changes in color, and, in the rabbit's ear, by direct observation of the vessels.

Adrenalin chloride (Parke, Davis & Co.) made up with 0.9 per cent sodium chloride to a dilution of 1:250,000 was injected intravenously at a constant rate through one arm of a Y tube. To the other arm of the Y, a flask of normal saline was attached, so that the subject was receiving a constant slow injection of fluid during the entire test and was unaware of the nature of the solution. From 2 to 6 cc. of solution were injected per minute containing an amount of adrenalin equivalent to from 0.0001 to 0.0003 mgm. per kilo per minute. The rate of the injection was governed by the degree of reaction as estimated from the pulse rate, blood pressure, and subjective symptoms. No untoward reactions were encountered.

In a second series of experiments, the hypoglycemia resulting from the administration of insulin was used to stimulate secretion of the adrenal glands, since it could be readily controlled and did not interfere with the temperature determinations. Blood was taken from the fasting subject for sugar determinations by the Folin and Wu (1929) method and 15 to 30 units of insulin (Eli Lilly & Co.) were then injected subcutaneously. A second blood sample was taken when symptoms of hypoglycemia became apparent and immediately thereafter 200 cc. of sweetened orange juice were given. After subsidence of the reaction a final estimation of the blood sugar level was made.

Experiments were made on cats after sympathectomy of one paw and on rabbits after total denervation of one ear (Grant, Bland, and Camp, 1932) before and after adrenal exclusion. Prior to the second test, one adrenal

was removed and the other denervated by section of the splanchnic nerves and removal of the upper abdominal sympathetic chain. Between 1 and 3 units of insulin per kilo were injected intraperitoneally and blood was taken from the ear vein for glucose determinations by the Folin (1932) method. Samples of blood were taken before injecting the insulin, during the reaction, and after administration of 50 cc. of 10 per cent glucose intraperitoneally.

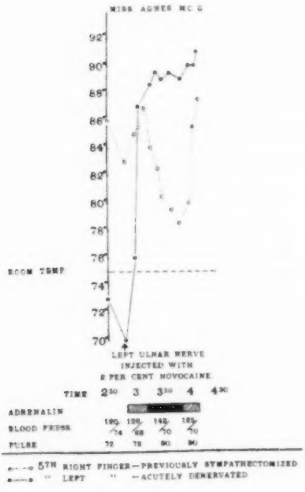

Fig. 1

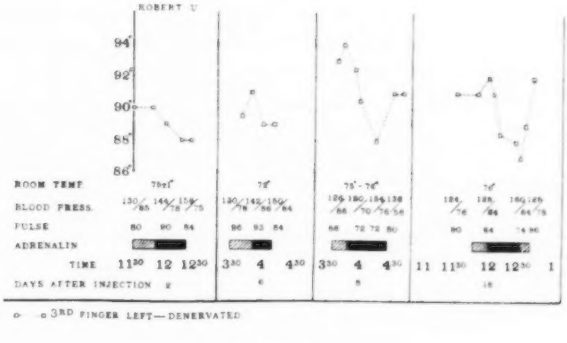

Fig. 2

Fig. 1. The surface temperatures of the 5th finger of both hands upon intravenous injection of adrenalin in a case of early Raynaud's disease. Right cervico-dorsal sympathectomy 3 months previously. Broken line, sympathectomized; solid line, acutely denervated. Injection of left ulnar nerve at 2:48. In this, as in the following figures, cross hatchings denote injection of saline; solid block, adrenalin 1:250,000.

Fig. 2. The surface temperatures of the left 3rd finger upon intravenous injections of adrenalin. Bronchial asthma. Left paravertebral alcohol injection 2 days before first record.

Results. Figure 1 shows the drop in surface temperature in an extremity where the blood vessels had been rendered sensitive to adrenalin by previous sympathectomy. This decrease in surface temperature was associated with cyanosis of the fingers. The temperature did not fall in the left little finger, which was acutely denervated by novocaine anesthesia of the ulnar nerve. This patient was suffering from early Raynaud's disease. Three months previously the right inferior cervical, first and second thoracic sympathetic ganglia had been resected (White, Smithwick, Allen, and Mixter, 1933). The typical response on anesthetization of the ulnar nerve

in Raynaud's spasm is seen in the rise of temperature shown in figure 1. Evidently, a dose of adrenalin which fails to lower the temperature of the acutely denervated area causes a definite drop in the sensitized region where the post-ganglionic neurons have degenerated. A similar response was obtained in each of seven other patients who were studied,—cases of Raynaud's disease, and also of asthma and epilepsy in which the peripheral

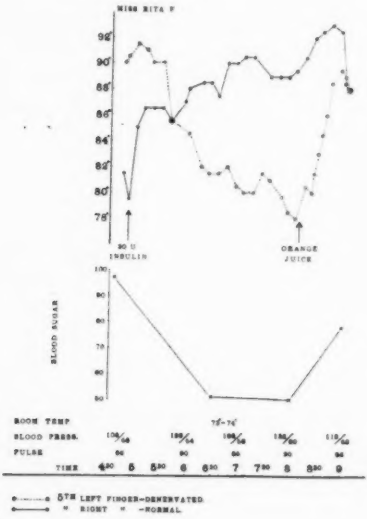

Fig. 3

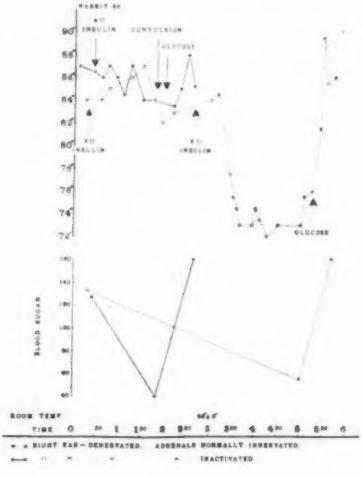

Fig. 4

Fig. 3. The surface temperatures of the 5th finger of both hands, during insulin hypoglycemia. Epilepsy. Left cervico-dorsal ganglionectomy three weeks previously. Broken line, sympathectomized; solid line, normally innervated. Insulin, 30 units, injected subcutaneously at 4:50. Orange juice with sugar given at 8:10. Lower curve gives blood sugar level.

Fig. 4. The surface temperatures of the sympathectomized right ear and the blood sugar levels of a rabbit during insulin hypoglycemia. Broken lines before, solid lines after removal of the left and denervation of the right adrenal. The arrows indicate the times of injections of insulin and glucose intraperitoneally and the convulsion experienced by the rabbit after the adrenal operation.

circulation was presumably normal (Smithwick, Freeman, and White, 1934). Cyanosis, however, was observed only in the cases of Raynaud's disease.

The time interval between sympathectomy and the occurrence of sensitization to adrenalin is depicted in figure 2. This patient received an injection of alcohol into his left paravertebral ganglia (White, 1930) which gave every evidence of accomplishing a complete sympathectomy of the left arm. A unilateral Horner's syndrome, elevated surface tempera-

ture and absence of sweating and pilomotor activity were present. Injections of adrenalin on the 2nd and 6th days following the paralysis gave no indication of sensitization. On the 8th day, however, under similar conditions with the same dosage of adrenalin, a definite drop of temperature occurred. A similar lowering of temperature was obtained on the 18th day.

This sensitization is present not only for injected adrenalin, but also for adrenin secreted in response to insulin hypoglycemia. Figure 3 shows that as the blood sugar falls the surface temperature of the sensitized extremity decreases, while, in contrast, the temperature of the normal extremity actually rises. On administration of orange juice and sugar the surface temperature rises to its initial level. In each of the 5 subjects who received insulin injections a similar response was obtained.

Figure 4 demonstrates a totally different reaction after excluding the adrenals. During insulin hypoglycemia in a rabbit (blood sugar 50 mgm. per cent) with the right ear denervated and the adrenals intact, the surface temperature fell 14°F. In the same animal after denervation of one adrenal and removal of the other, a blood sugar of 40 with the animal in convulsions was accompanied by a reduction in the temperature of the denervated ear of only 3°F. This observation was also confirmed in cats.

Discussion. Increase in sensitivity of the pupil to adrenalin after removal of the superior cervical ganglion was first observed by Meltzer and Auer (1904). This sensitization was found by Elliott (1905) to apply to all structures innervated by the sympathetic nervous system. He found that it appeared between one and two weeks after denervation. Our observations on the sensitization of the sympathectomized blood vessels in man and the interval between denervation and the onset of sensitivity (see figs. 1 and 2) are quite in keeping with the results obtained in animals by Elliott.

Since the sensitized blood vessels constrict when adrenalin is injected, their similar reaction to adrenin secreted in response to insulin hypoglycemia (see fig. 3) is to be expected. The increase of adrenin in the blood in hypoglycemia was first discovered by Cannon, McIver and Bliss (1925) in cats. They used the denervated heart as an indicator. In man, the administration of insulin has been reported to increase the adrenin content of the blood assayed biologically outside of the body (Brandt and Katz, 1933). The experiments here presented, in which the denervated blood vessels were used as an indicator of medulli-adrenal secretion, fully substantiate observations on the cat since they demonstrate the active secretion of adrenin in the intact human body. Control tests with insulin in man, with the adrenal mechanism eliminated by spinal anesthesia, were attempted. These experiments proved to be unsatisfactory since the level of the sympathetic paralysis was difficult to define exactly and the duration of anesthesia was too short. The animal and human experiments showed

a striking similarity of reaction to hypoglycemia up to the point of adrenal inactivation. The response in animals after adrenal inactivation (see fig. 4) therefore served as a control for the human cases, indicating that the drop in surface temperature during hypoglycemia (see fig. 3) was in reality due to secreted adrenin.

CONCLUSIONS

The blood vessels of the human extremity become more sensitive to adrenalin after sympathectomy.

This sensitization appears between 6 and 8 days following paralysis of the regional sympathetic nerves.

The sensitized digital arterioles have been utilized as an indicator of circulating adrenin. During insulin hypoglycemia, increased medulli-adrenal secretion is shown by striking vasoconstriction in sympathetomized human extremities.

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ON THE NERVOUS CONTROL OF LACTATION

HANS SELYE

From the Department of Biochemistry, McGill University, Montreal

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It is well known that lactation stops very soon after the young are weaned, and that in cows and goats the secretory activity of the mammary gland lasts only as long as the animals are milked regularly. Two alternative explanations have been offered; the first being that the act of suckling reflexly maintains lactation, and the second and more familiar being that the accumulation of milk in the gland has a detrimental effect on the secretory epithelium. Our knowledge of the nervous factors involved is far from complete; early experiments involving cutting or stimulating the nerves of the mammary gland were inconclusive. Basch (1906, 1909), who reviews this early literature, found in dogs that mammary transplants might secrete after parturition; Stricker (1929) obtained similar results in rabbits, Goltz and Ewald (1896) found that almost complete removal of the spinal cord did not interfere with either pregnancy or lactation in dogs; according to Cannon (1930, 1932) and Bacq (1932), sympathectomy may inhibit lactation in cats and rats, but does not do so invariably nor immediately. Kuramitsu and Loeb (1921) ligated the nipple of one mammary gland in guinea pigs and found that this gland involuted as if the animal had been weaned, while the other continued to secrete; they concluded that the involution of the gland was due to the accumulation of the products of secretion. However, the experiments to be reported in this paper suggest that, unless the guinea pig differs greatly from the rat, Kuramitsu and Loeb would have obtained very different results had they examined the glands at an earlier stage after the operation.

It seemed at first quite difficult to obtain decisive experimental evidence on this point, since suckling necessarily leads to the removal of the products of secretion, and weaning to their accumulation in the gland. Eventually the following experiment was devised: the main milk duct was cut at each of the nipples of seven lactating rats, three days after parturition. The skin incisions were made at some distance from the nipple and the main galactophore was approached through a channel made in the subcutaneous tissue, so that all possible injury to the nipples was avoided. After the operation the mothers were returned to their litters; as none of the nipples gave milk, the young nursed them all to the same extent. In order to

keep the young alive they were placed with a normal litter on alternate days, the latter's own young being transferred on these days to the experimental animal. Thus the operated animals were continuously exposed to the suckling stimulus, without emptying of the glands.

In a control series the litters were removed from ten mother rats on the third day of lactation, in order to see how soon the mammary gland would show signs of involution after the stimulus of suckling ceased. In two of these controls the galactophores were cut as in the experimental series—a further check which later proved to be unnecessary. The animals of both series were killed at various intervals, 3 to 14 days after the beginning of the experiment. Examination showed that in the entire first series, even

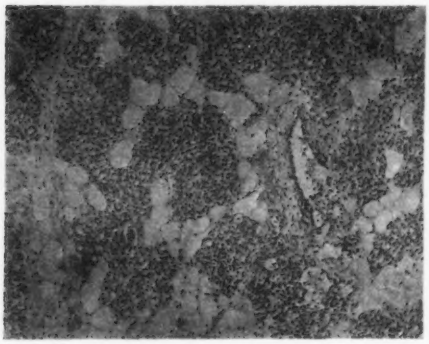

Fig. 1

Fig. 2

Fig. 1. Mammary gland of a rat 8 days after her litter has been removed. The alveolar structure of the gland has completely disappeared, and only solid epithelial cell nests are left.

Fig. 2. Mammary gland of a rat 14 days after transcision of all the milk ducts. Alveoli well preserved and turgid with milk. At the point marked by a cross, milk has forced its way into the stroma.

those killed after 14 days, the mammary glands were turgid with a large amount of milk, while in the second series the glands contained no milk after 3 to 5 days, and in 6 to 8 days the alveoli of the gland began to disappear (see fig. 1). When the two series of glands are compared histologically the difference is obvious. Throughout the first series the alveoli are well preserved and filled with milk; in many places the accumulation of milk is so great that the alveolar wall gives way and the milk forces its way into the stroma (see fig. 2). This escape of milk between the connective tissue fibres of the gland is not, strictly speaking, pathological, since it may be seen to occur, though less markedly, in normal mother rats on the first or second day after weaning: that is, at a time when milk is still

secreted but is no longer removed from the gland. In the second series of animals, however, the alveolar structure disappeared in six or eight days, and thereafter the entire parenchyma consisted of solid epithelial cell nests.

These experiments show beyond doubt that the afferent stimulus of suckling leads to the secretion of milk, though the pathways through which this is accomplished remain obscure. The centrifugal part of the mechanism is somewhat clearer. It is known that the anterior hypophysis contains a substance, the "prolactin" of Riddle, Bates and Dykshorn (1932), capable of initiating secretion in developed mammary glands, and Collip, Selye and Thomson (1933) have shown that removal of the hypophysis stops milk secretion; the above-mentioned experiments on mammary transplants indicate that the action of this hormone on the mammary glands is independent of their nerve supply. It is, therefore, not unlikely that the act of suckling stimulates the production of prolactin by the pituitary just as the act of copulation, in some species at least, stimulates the production of the ovary-stimulating hormone; and it has been possible to obtain evidence which is favorable to this hypothesis, and which at least rules out the possibility that the effect of suckling is merely local.

In eight lactating rats, three days after parturition, the main galactophores were tied as before on one side of the body, while all the nipples on the other side of the body were excised so as to make suckling from them impossible (excision of *all* nipples, like removal of the litter, leads rapidly to involution of the glands). Examination of the mammary glands 14 days after these operations showed that active milk secretion was still present on *both* sides of the body. In a further series of seven animals, the nipples were excised on one side of the body while the glands on the other side were not touched; it was again observed that rapid involution did not occur in the glands operated upon. It is clear that the stimulus of suckling at one set of nipples may maintain active secretion in glands not exposed to suckling.

SUMMARY

The act of suckling maintains the mammary glands of the rat in the stage of active secretion over long periods, even though the galactophores are cut and the milk cannot be removed. Suckling at one set of nipples may maintain active secretion in other glands where suckling has been prevented by excision of the nipples.

The physiological involution of the mammary gland after weaning is thus conditioned by the withdrawal of the nervous stimulation of suckling rather than by the accumulation of the products of secretion in the gland. It is probable that the act of suckling stimulates the production of the hypophyseal hormone, prolactin.

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THE HYDROGEN-ION CONCENTRATION OF MAMMALIAN VOLUNTARY MUSCLE UNDER VARIOUS CONDITIONS

CARL VOEGTLIN, R. H. FITCH, HERBERT KAHLER AND J. M. JOHNSON

From the Division of Pharmacology, National Institute of Health, U. S. Public Health Service

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In a paper (Voegtlin, Kahler and Fitch, to be published elsewhere) a method will be described for the estimation of the hydrogen-ion concentration of the tissues in living animals. In the present report data will be presented on the application of this method to voluntary muscle. This tissue was chosen for two reasons. First, intensive research has revealed the occurrence of several biochemical reactions which may influence the acid-base balance of muscle and, therefore, this knowledge should assist in the interpretation of any observed changes in pH. Second, estimations of the pH of muscle, when related to the pH of certain malignant tumors, are of interest in an attempt, along biochemical lines, to elucidate the reasons for the destructive action of cancer cells on infiltrated muscle tissue.[1]

Most of the work was done on rats, though a few observations were also made on cats and rabbits. Rats were chosen in order to correlate the pH of rat muscle with the pH of malignant rat tumors. The rats came from an inbred stock of this laboratory and were deprived of food, except water, for 18 to 24 hours before the experiments were begun. They received an intraperitoneal injection of approximately 0.6 cc. of a 0.5 per cent solution of pentobarbital per 100 gram body weight. This anesthetic, when injected intraperitoneally, produces its maximum effect in about 15 to 20 minutes. If, at the end of this period, the animal was not lightly anesthetized, a second or even third smaller dose (0.2 cc.) was given subcutaneously. The aim was to obtain a light degree of anesthesia, just sufficient to completely immobilize the animal. If, during the course of an experiment the animal showed signs of coming out of the anesthesia, a small dose was again given. One hind leg with cut skin was immersed in Ringer solution and the glass electrode was inserted through a skin incision into the muscles of the other upper hind leg (M. gluteus). The electrical circuit was then immediately completed by moving the agar bridge from the saturated KCl solution to the Ringer solution and throwing the electrometer

[1] Data on this subject will be published elsewhere.

switch so as to insert this instrument into the circuit. Working very rapidly, it was feasible to make the first E.M.F. reading about 15 seconds after the insertion of the electrode into the tissue. Similar measurements were then made at intervals of 1 or 2 minutes for the first 10 minute period, and every 5 or 10 minutes thereafter, unless for special reasons it was desirable to make readings more frequently. A record was also kept of the rate and character of respiration. The results will be discussed in the following pages.

A. pH of resting muscle under normal physiological conditions. After the technic had been perfected the pH of rat muscle was studied in 33 lightly anesthetized, normal animals. Chart 1 illustrates the principal features of three typical experiments. The first E.M.F. reading was made about 1 minute after insertion of the electrode. At this time the pH in experiment I was 7.56, in II 7.58, and in III 7.56. This close agreement is a

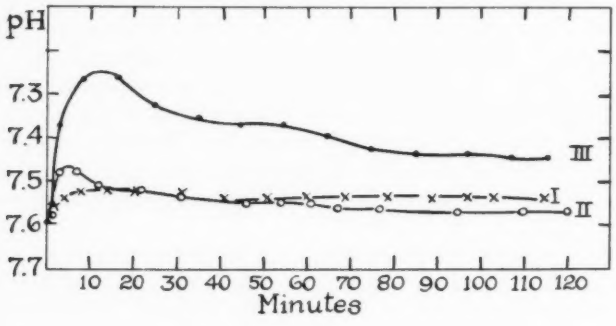

Chart 1

coincidence as considerably greater variations in initial readings occur as a rule. The usual course of events during the first 5 to 10 minutes is a gradual decrease in pH, followed by a slower rise and finally the pH reaches an equilibrium value which is maintained with minor deviations (± 0.01 pH) for an hour or longer, provided the physiological conditions are relatively unchanged. Part of the initial acid drift is undoubtedly due to the rapid production of acid resulting from the injury attendant upon the insertion of the electrode into the tissue. This explanation is supported by the following facts. We have found that electrodes having a straight and smooth sealed point and narrow diameter produce relatively slight drifts, such as are illustrated by curves I and II, and electrodes of such construction are satisfactory for the estimation of pH. If, on the other hand, the end of the glass capillary has a curved instead of a straight seal, or if the electrode has a wide, conical capillary, then the acid drift is more marked

and the establishment of an equilibrium pH requires a very long time. Curve III was obtained by means of such an unsatisfactory electrode. It will be noted in this case that even after 115 minutes the tissue had not recovered from the injury effect.

In table 1 we have compiled the initial, lowest and equilibrium pH and the time after the insertion of the electrode at which these values were obtained in 12 typical experiments made during the spring of 1933. Five different glass electrodes were used in this series. It will be noted that the initial pH shows considerable variation, the highest values being obtained if the first reading is made very soon after the electrode insertion. We are

TABLE 1

Initial, lowest and equilibrium pH of resting rat muscle in the living animal under normal physiological conditions

DATE	INITIAL pH	LOWEST pH	EQUIL. pH
4/28	7.55 (1 min.)	7.43 (8 min.)	7.50 (52 min.)
5/5	7.67 (1 min.)	7.46 (9 min.)	7.52 (49 min.)
5/3	7.56 (1 min.)	7.47 (5 min.)	7.53 (56 min.)
5/4	7.63 (1 min.)	7.48 (9 min.)	7.54 (77 min.)
5/15	7.76 (15 sec.)	7.48 (7 min.)	7.60 (52 min.)
5/12	7.67 (15 sec.)	7.48 (11 min.)	7.60 (77 min.)
5/11	7.74 (17 sec.)	7.47 (5 min.)	7.60 (54 min.)
5/19	7.62 (1 min.)	7.51 (14 min.)	7.56 (71 min.)
5/20	7.56 (30 sec.)	7.52 (8 min.)	7.54 (41 min.)
4/14	7.58 (1 min.)	7.48 (3 min.)	7.56 (67 min.)
4/7	7.52 (1 min.)	7.48 (5 min.)	7.54 (59 min.)
3/15	7.56 (1 min.)	7.39 (10 min.)	7.52 (83 min.)
Average.....		7.47 (8 min.)	7.55 (60 min.)
Max.....		7.52	7.60
Min.....		7.39	7.50

of the opinion that these initial readings are subject to various errors and should be considered merely as rough approximations. First of all we have found in calibrating the electrodes that even at constant temperature the electrode does not instantly come into equilibrium when it is changed from one buffer to another, even though it is washed with distilled water before immersion into the second buffer. Therefore, it is to be expected that a short time is also required for the electrode to adjust itself to the tissue. A second factor which may play a rôle is the time required for the electrode to reach the temperature of the tissue. This error probably is of minor significance in the case of electrodes having a very small temperature correction. A third factor is the variable electric charge given to the

animal in the unavoidable handling of the animal during the insertion of the electrode. In all events, we do not place great reliance in the accuracy of E.M.F. readings taken within about one minute. The equilibrium values given in table 1 are, however, surprisingly consistent and evidently dependable. The average pH is 7.55 and is reached in an average of 60 minutes. The deviation is ±0.05 pH. In a further series of 24 experiments, which are not included in the table, the average equilibrium pH was 7.55. Our results, using the 12 selected runs, give pH = 7.55 with a probable error $\left(e = \pm .67 \frac{\sqrt{\Sigma (v - m_i)^2}}{n(n-1)}\right)$ of ±0.0067 pH. A total of 36 runs which we have made on muscle, neither discarding any measurement nor weighting seemingly preferred measurements, gives a pH of 7.55, with a probable error of ±0.0082 pH unit. We may conclude, therefore, that a pH of about 7.55 is characteristic of the rat muscle in the living, lightly anesthetized animal, whose respiration is adequate to insure efficient pulmonary ventilation and consequently an adequate supply of oxygen to the tissues.

Another point of interest is the magnitude of the injury effect in terms of pH, when electrodes of the best construction and satisfactory calibration are used. If we assume that on reaching the equilibrium pH, the part of the tissue which is in contact with the electrode has a normal blood circulation and that any slight damage to the tissue has been overcome, then the difference in the equilibrium pH and the lowest pH reached previously should give the magnitude of the injury effect in terms of pH. According to table 1, the average reduction in pH resulting from injury is 7.55 − 7.47 = 0.08 pH. From the work of Fletcher and Hopkins (1907) and subsequent workers, there can be little doubt that this acid injury drift is largely due to the local production of lactic acid. This acid is sufficiently strong (dissociation constant pK. 3.85) to overcome temporarily the buffer capacity of the tissue. A similar acid drift attributable to tissue injury we have observed also in other tissues with an active metabolism. Curves similar to those of chart 1 were also obtained in experiments on the skeletal muscle of cats and rabbits.

B. Effect of deep pentobarbital anesthesia. In numerous experiments we have found that the equilibrium pH does not change appreciably when the animal is allowed to recover from the anesthesia sufficiently so as to show slight body movements and then is given a small dose of pentobarbital sufficient to abolish these movements. It would appear, therefore, that slight variations in depth of anesthesia, provided this is always light, have no influence on muscle pH. In order to ascertain whether deep or fatal anesthesia will bring about a shift in pH, a few experiments were made in which, after equilibrium had been reached under light anesthesia, a large dose of pentobarbital was injected. The results may be summarized by

stating that the muscle pH showed a gradual decrease coincident with a decrease in respiratory rate and depth. In one experiment the respiratory rate during light anesthesia was 68 per minute. A dose of 8 cc. 0.5 per cent pentobarbital was injected. The respiratory rate gradually decreased and reached zero, 15 minutes after the injection, at which time the muscle pH had dropped 0.06 pH from the equilibrium value.

C. Effect of deficient respiration. As a rule, there is a tendency for the pH of muscle to decrease when the respiration is depressed. However, minor fluctuations in pulmonary ventilation do not seem to produce any effect.

The following experiment illustrates the striking decrease in the pH of muscle resulting from a gradual and severe respiratory depression. The rat received the ordinary doses of pentobarbital and the respiration was normal in character during the first 30 minutes after the electrode had been inserted into the muscle. The initial pH was 7.55, followed by the usual acid drift (pH 7.47), with recovery to 7.55. During the next interval (30 to 80 minutes from start) the respiration gradually became more shallow and decreased in rate from 44 to 20 per minute and finally ceased for a short time. The muscle pH decreased gradually from 7.55 to 7.42. During the following interval (80 to 147 minutes from start) the respiration became still more depressed, the rate varying from 18 to 20 per minute. The muscle pH declined from 7.42 to about 7.30. After another short period of cessation of respiration, the latter became extremely shallow though maintaining its previous rate. During this period until the respiration ceased again (228 minutes from start) there was a further gradual acid drift to pH 7.06. The muscles were then frozen *in situ* with solid CO_2 and analyzed for lactic acid by the method of Friedemann, Cotonio and Shaffer as improved by Friedemann and Graesser (1933). The muscle contained 111 mgm. per cent lactic acid and the liver 97 mgm. per cent, whereas the average figures for normal muscle and liver are, according to our analyses, as follows: muscle 36 mgm. per cent, liver 15 mgm. per cent. We may conclude, therefore, that severe depression of respiration will cause an acid drift in skeletal muscle, which is accompanied by an increase of over 100 per cent in the lactic acid content of the tissue.

D. Effect of hemorrhage. A considerable reduction in the O_2 carrying capacity of the blood following severe hemorrhage may possibly produce a change in tissue pH. In order to test this question the following experiment was carried out.

A rat was lightly anesthetized and sufficient time was allowed for the electrode to assume an equilibrium potential, i.e., 7.525 pH. The respiration was normal in character and 72 to 76 per minute. Hemorrhage was then produced by severing the tail and immersing the cut end into a beaker containing warm water, so as to promote bleeding. The beaker rested on

the insulated animal board. The bleeding continued for 32 minutes and the animal had an anemic appearance. Ten minutes after the beginning of the bleeding the respiration became irregular, then increased in rate to 120, and finally became asphyxial. There was no change in muscle pH during the first 15 minutes, but then the pH decreased gradually from 7.52 to 7.45, simultaneously with the appearance of asphyxial respiration. After the bleeding stopped (32 minutes from start) the muscle pH maintained itself at 7.45 with minor fluctuations during the subsequent 50 minutes, the respiratory rate changing irregularly from 64 to 160 per minute.

This experiment indicates that severe hemorrhage caused an acid shift of only 0.075 pH. Stimulation of respiration evidently partly compensated for the decrease in O_2 carrying capacity of the blood and thus minimized the tissue acidosis. More exact studies of the influence of hemorrhage on pulmonary ventilation, blood hemoglobin, and muscle pH could be made on larger animals.

E. Effect of the parenteral injection of sugars. The experiments under this heading were done in order to ascertain if a great increase in blood sugar would sufficiently increase the lactic acid production in muscle so as to cause a decrease in pH. The sugars used were of the highest purity and were kindly prepared for us by Prof. Claude S. Hudson and his collaborators. In each experiment sufficient time was allowed for the electrode to reach and maintain a steady potential ($1\frac{1}{2}$ to 2 hours from time of electrode insertion). The sugar was then injected intraperitoneally in a dose of 600 mgm. per 100 grams body weight. Special care was taken not to disturb the electrode. The action of the following sugars was tested: glucose, fructose, galactose, maltose and lactose. At the end of the experiments the lactic acid content of the muscle was determined. The results may be summarized by stating that there was a definite tendency for the pH to decrease and the lactic acid to increase after injections of glucose and fructose, whereas the other sugars had no effect, or only a slight one. For instance, an experiment with glucose showed a gradual decrease in muscle pH of 0.06 during the first hour following the injection and the muscle contained 51 mgm. per cent of lactic acid 108 minutes after the injection. In two experiments with fructose the decrease in pH was 0.07 and 0.11 respectively, and the lactic acid content 74 and 73 mgm. per cent respectively. Apparently the slight decrease in pH is accompanied by and may be due to the increased formation of lactic acid in muscle. It should be remembered that these results were obtained with massive doses of sugar and, therefore, smaller doses probably would be without effect.

F. Effect of various lethal agents. We already have called attention to the simultaneous decrease in respiration and muscle pH following the injection of a fatal dose of pentobarbital. The decrease in pH continues

after death. Further experiments were made to determine the influence of other lethal agents on the muscle pH curve. Again sufficient time was allowed for attainment of an equilibrium pH before the lethal agent was administered.

One animal then received a subcutaneous injection of KCN (3 cc. of a 0.01 molar solution per 100 g.). There followed a gradual increase in pH of about 0.1 during the next 6 minutes, which was apparently caused by the stimulation of respiration (over ventilation) by the cyanide. The subse-

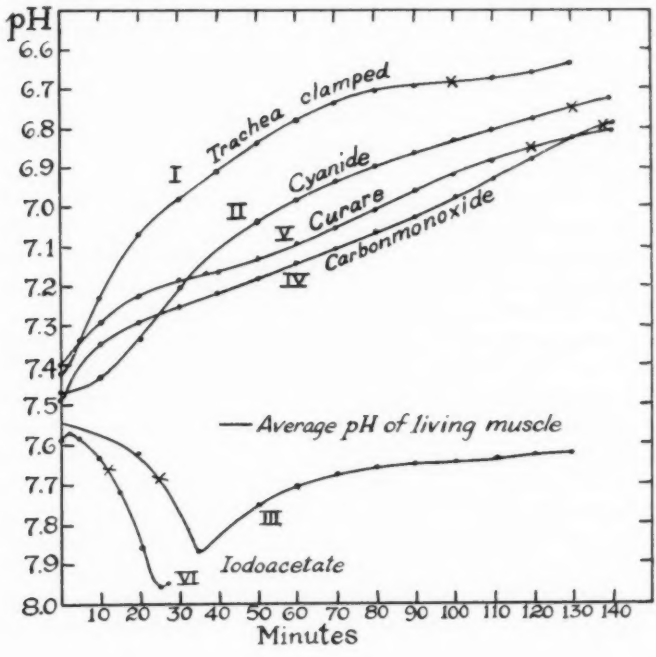

Chart 2

quent gradual depression of respiratory activity coincided with a decline in pH and after the respiration had permanently ceased the rate of decline in pH increased during the following 10 minutes, after which it slowed up. Another experiment with KCN showed a decrease in pH of 0.17 at the time of cessation of respiration. The post-mortem pH curve is shown in chart 2, curve II. The cross mark on the curve indicates that rigor mortis had appeared.

A similar result was obtained from the subcutaneous injection of a lethal dose of curare, chart 2, curve V.

In another experiment the animal was killed with illuminating gas (rich in CO) by placing the head of the animal into a thistle tube which was connected with a gas pipe. Death occurred in 1½ minutes and was followed immediately by a continuous decrease in muscle pH (chart 2, curve IV).

Another procedure used to cause death was to clamp the trachea. This also led to a fairly rapid decline in muscle pH (chart 2, curve I).

The principal conclusion to be drawn from these experiments is that the postmortem pH curve of muscle is characterized by a rapid decrease of the normal alkaline muscle pH which gradually passes over into the acid range. The cause of this drift is undoubtedly due primarily to the interruption of the blood circulation, which is followed 1, by exhaustion of the oxygen supply of the muscle, and the establishment of anaerobic conditions, and 2, the prevention of removal of acidic metabolism products, such as CO_2 and lactic acid.

A totally different result was obtained when the animals were killed by an intravenous injection of monoiodoacetic acid (15 mgm. per 100 gm. body weight, neutralized with Na_2CO_3). Lundsgaard (1930a) and subsequent investigators had found that this interesting substance prevents the formation of lactic acid during muscular fatigue and that the muscles pass over into a rigor without a coincident increase in lactic acid. Lundsgaard also observed that deep anesthesia prevents the rapid appearance of rigor after the injection of iodoacetate. Several experiments were made, therefore, on lightly anesthetized rats. After the electrode in the muscle had maintained an equilibrium value for a sufficient time, iodoacetate was injected into a tail vein. This caused a rapidly increasing depression of respiration and respiratory failure in from 5 to 8 minutes. During this period the muscle pH either remained unchanged or had a slight tendency to decrease. This was followed by a gradual increase in pH and the appearance of rigor about 15 minutes after death. Typical postmortem curves are included in chart 2, curves III and VI. It will be noted from these curves that the alkaline drift is very striking and persists for about 25 to 35 minutes after death. The muscle used in the experiment illustrated by curve VI was analyzed immediately after the last estimation of pH (i.e., 7.95) and showed 46 mgm. per cent lactic acid.

In another experiment the muscle pH had risen from 7.52 before iodoacetate to 7.87 35 minutes after death. The muscle was in rigor and contained only 17 mgm. per cent lactic acid.

After having reached the maximum pH a gradual decrease occurs, but even 2 hours after death the pH of the poisoned muscle may still be above that of the normal resting muscle (chart 2, curve III). We have failed to observe such alkaline drifts in several experiments where death occurred after a subcutaneous or intraperitoneal injection of the same amount of

iodoacetate. These negative results are probably accounted for by assuming that death occurs before a sufficient amount of the poison has reached the muscle. In such instances the pH shows the usual postmortem acid drift and analysis of the muscle reveals an increase in lactic acid above normal. Increase in depth of anesthesia also decreases or prevents the alkaline drift and the occurrence of rigor in the iodoacetate muscle. Evidently normal muscular tone is a prerequisite for the rapid production of rigor and the alkaline drift following intravenous injections of iodoacetate.

How can these alkaline drifts be explained on the basis of our present knowledge of muscle chemistry? Theoretically, they may be due either to a diminution in acid equivalents, an increase in base equivalents, or a combination of the two possibilities. As to the diminution in acid equivalents, there is at present no evidence supporting this possibility. The lactic acid content of the muscle is certainly not diminished sufficiently to account for the alkaline drift. However, Lundsgaard (1930a) has shown that creatine-phosphoric acid breaks down to creatine and phosphoric acid in the poisoned muscle during contraction. Moreover, Meyerhof and Lohmann (1928) made electro-titration curves of creatine-phosphoric acid and of the products of its hydrolysis and found that creatine-phosphoric acid has little or no buffering action between pH 7 and 9, but that hydrolysis of this substance sets free base equivalents. Therefore, the breakdown of creatine-phosphoric acid in the iodoacetate muscle may partly account for the alkaline drift. Another possibility is the liberation of base equivalents resulting from the hydrolysis of adenyl-pyrophosphoric acid in the poisoned muscle (Lundsgaard, 1930b). The liberation of ammonia from adenyl-pyrophosphoric acid takes place even before the appearance of rigor. Ammonia is a strong base and may very well be partly responsible for the alkaline drift. The gradual decrease in pH, after the maximum has been reached, is probably due to a partial resynthesis of creatine-phosphoric acid and the accumulation of lactic acid. That lactic acid may accumulate in considerable amount in the poisoned rat muscle *in situ* long after death from an intravenous injection is shown by an experiment in which the muscle was in rigor with a pH of 7.84. The pH then gradually declined and reached a value of 7.22 at the end of 207 minutes at which time the lactic acid content was 117 mgm. per cent.

Data which will not be given due to lack of space indicate 1, that there is a rough correlation between pH and lactic acid content in the intact muscle after death. Furthermore, rigor appears at a time when the pH has reached 6.8 to 6.7 and the lactic acid shows a sixfold increase above normal. The lowest pH values (6.25–6.21) were obtained in dead animals which had been kept at a temperature of 6°C. for one or two days. These muscles showed about a twelvefold increase in lactic acid above normal.

Discussion. We may now refer to the work of other investigators who have attempted to estimate the pH of muscle. Michaelis and Kramsztyk (1914) determined the pH of extracts of normal, resting muscles of cats and guinea pigs by means of the hydrogen electrode and found pH 6.02 for cat muscle and pH 6.05 for guinea-pig muscle. These figures were obtained at a time when the extreme errors, which may be introduced by faulty technic, were not properly appreciated. The animals were bled to death, the tissue ground in a mortar at room temperature, shaken with distilled water for one-half hour, centrifuged, and the supernatant fluid used for the electrometric estimations. The acid values thus obtained are explained by postmortem acid production during the preparation of the extracts. We have found that when rat muscle is removed from the living animal and is rapidly minced with scissors at a temperature of 25°C. the pH estimated by the glass electrode 5 minutes after removal of the tissue from the animal has already fallen to about 6.5 and the decrease continues rapidly, reaching pH 6 in about 30 to 40 minutes.

Furusawa and Kerridge (1927) attempted to avoid lactic acid production by freezing the muscle in liquid air, followed by grinding in the cold room and estimation of the pH by the glass electrode (cupshape) at a temperature slightly above zero. The gastrocnemius of cats, deeply anesthetized with ether and chloralose, gave values for resting muscle of pH 7.04, probable error ± 0.03; after fatigue pH 6.26, ± 0.07; and in rigor pH 6.02, ± 0.07. These authors then proceeded to calculate the pH for body temperature. In our opinion their temperature correction is not on a secure basis. For this reason and in the absence of any conclusive proof showing that all chemical changes are eliminated during the preparation of the material, we regard the above figures as approximate and considerably lower than the figures for intact muscles. We attempted to apply the technic of Furusawa and Kerridge to rat muscle, but met with great difficulties from rapidly drifting potentials.

Mackler, Olmsted and Simpson (1930) determined the pH of muscle from decapitate cats, excised and frozen in liquid air, ground, distilled water added, and centrifuged. The supernatant fluid gave with the quinhydrone electrode an average pH of 7.42 at 10°C. and pH 7.41 at 25°C. The authors point out that the high lactic acid found in these samples indicates abnormal conditions in the tissue before and during the sampling. There is also some question as to the reliability of the quinhydrone electrode for measuring pH in tissue extracts.

Carlström, Ege and Henriques (1928) estimated the pH of rabbit muscle according to the technic of Furusawa and Kerridge, with the exception that the measurement with the glass electrode was made at 18°C. They obtained an average value of 7.04.

Meyerhof and Lohmann (1926) estimated the pH of extracts of resting

frog muscle by the quinhydrone electrode as about 7.25 to 7.35. In a later paper Lipmann and Meyerhof (1930) admit that estimations of pH made on muscle extracts do not yield reliable figures for the intact fresh muscle *in situ*.

We conclude, therefore, that the capillary glass electrode method, when carefully applied to the living muscle *in situ*, avoids several objections which can be raised against the estimation of the pH of muscle made on macerations or extracts.

CONCLUSIONS

The capillary glass electrode method has been used for the estimation of the pH of intact mammalian skeletal muscle in the living animal with the following results:

The insertion of a properly constructed electrode into the muscle of lightly anesthetized rats is followed by a relatively small acid injury drift and a slow return to an equilibrium value. The latter is maintained with only slight deviations for an hour or longer, providing the physiological condition and particularly the respiration remain unchanged. The average equilibrium value is about pH 7.55 (probable error ± 0.008), a range which is somewhat higher than that reported in the literature for the pH of normal rat blood.

Severe depression of the respiration produces an acid drift. Extensive hemorrhage also causes a decline in pH.

The intraperitoneal injection of large doses of glucose or fructose causes a moderate decrease in pH.

Death caused by asphyxia, cyanide, carbonmonoxide or curare is followed by a gradual decrease in pH. Rigor mortis appears when the pH has decreased to about 6.85 to 6.68, at which time the lactic acid content of the muscle has increased about six-fold above normal. The lowest postmortem pH observed is 6.21.

Death following intravenous injection of 15 mgm. per 100 grams body weight of iodoacetic acid is followed by a gradual rise in pH to as high as 7.96 and the appearance of contracture. This alkaline drift may be explained by the liberation of base equivalents from the breakdown of creatine-phosphoric acid and the liberation of ammonia from adenylpyrophosphoric acid. The subsequent decline in pH may be explained by partial resynthesis of creatine-phosphoric acid.

There is a rough correlation between pH and lactic acid content in the intact muscle after death.

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THE HEAT LIBERATED BY THE BEATING HEART

Eighth Communication: A New Method of Analyzing Waves of Heat Production; also a Study of Variability of Heat Production, Associated Directly with the Contractile Act

CHARLES D. SNYDER

From the Laboratory of Physiology, School of Medicine, The Johns Hopkins University

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The fact that the heart normally maintains its rhythmic activity uninterrupted during the whole of life leads one to suppose that this is only possible if all the conditions requisite for a contraction are fully primed before each beat. In current parlance of muscle physiologists this is equivalent to saying that the recovery processes due from one beat must be fully completed before the next beat begins. If this is so, then normally the heat liberated during the period following hard on active tension-change (the only period within the cardiac cycle when this recovery is possible) ought to bear a constant relation to the heat liberated during the period of active tension-change itself.

With some such thought as this, this series of investigations was begun in 1917. But the outcome to date has been to lead me to the view that heat production in heart muscle, both in absence and presence of oxygen, may be a very variable thing.

On the basis of work that had already been done by others on the effect of oxygen-lack on the contractility of muscle, I had assumed that the first wave of heat production in heart muscle, like the initial wave in skeletal muscle, would prove to be purely anoxidative; but, as I have shown, the turtle's ventricle when exposed to cyanogen and then to pure nitrogen gas for hours, the first as well as the second wave may be greatly reduced in the absence of oxygen (1928, p. 83). In another study on anoxybiosis on smooth muscle (in the experimental part of which I was aided by my students Messrs. N. L. Kaltreider and F. W. Light, 1929–30) I was able to show that the minimum heat production is obtained only when the oxygen withdrawal had been carried to the point where less than 0.001 per cent remains in the muscle chamber. Some of the smooth muscle preparations beat spontaneously and rhythmically; other preparations were thrown into contraction by stimulating their motor nerves. Direct electrical stimulation was never employed. These experiments made it quite clear that in

smooth muscle also the first wave, as well as the second wave, suffered marked reduction of magnitude. The conclusion was quite unavoidable that for cardiac and smooth muscle heat of oxidative origin may be liberated during the period of tension change as well as the period just following tension change, during the "initial" as well as during the "recovery" periods of muscle contraction.

But in smooth and cardiac muscle this reduction in the amount of heat liberated during the first and second phases does not always appear with the withdrawal of oxygen, and sometimes the amount liberated in oxygen-plenty is out of all proportion to the probable amount demanded by the immediate action of the muscle.

It is with these seemingly whimsical variations in heat production of muscle that the present paper deals especially. In the discussion an explanation is offered. Incidentally a new method of analyzing the graphic records of heat production is described.

1. Observation of the time relations of the waves of heat production to the phases of the heart's mechanogram is possible only in very slowly beating hearts, and I have found that the terrapin's heart, kept near the freezing point of water, meets this condition best. For then the normal rate of beat is about one per minute; and sometimes at this temperature, or in addition partial block may be induced, whereupon a single beat of the ventricle may last three minutes or longer. At this slow rate the cardiac muscle exhibits at least two chief waves of heat liberation during each beat. The first sets in promptly with systole then quickly subsides to be followed by the second which begins near the end of diastole, rises to a height less than that of the first wave, then gradually subsides, sometimes reaching the temperature level of the beginning systolic wave; but oftener, especially in the less slowly beating hearts, the decline of the second wave is interrupted by the onset and rise of the first wave of the next succeeding beat (1922). The first wave usually has the character of a simple wave, the second the character of a compound wave. Both auricular and ventricular muscles show both waves. In my earlier papers I regarded these two waves as the counterparts of the "initial" and "recovery" waves of heat production that then already had been described for skeletal muscle.

In the present experiments as in the former the hearts suspended in air beat against a carefully calibrated tension lever which was adjusted to register the slightest change of muscle length at maximum tension compatible with the maintenance of spontaneous rhythm. (The preparations were always allowed to contract only to their inner stimuli and no contractions were evoked artificially.)

The muscle chamber consists of a wide-mouth dewar jar of about 200 cc. capacity into which is fitted a thick rubber stopper which in turn carries supports for muscle fixation, leads to thermopile, and apertures for inlet

and outlet of fluid and gas. This chamber was submerged to within a half centimeter of the top in a water bath of melting ice stirred constantly with electric-motor driven stirrer. In the same bath two reservoirs of Ringer solution, connected one with oxygen the other with pure nitrogen by means of appropriate tubing and stop-cocks, enabled one to fill the muscle-chamber at will with one fluid or the other, or with only gas (oxygen of various mixtures or pure nitrogen, under pressure). The Ringer having been put in the bath much earlier (usually the day before) was cooled down to zero degrees; the muscle-chamber and its contents when filled with the Ringer solution thus could take on quickly the desired temperature, equilibrium good enough for recording thermocardiograms being established sometimes within a half-hour. The method described in 1929–30 using photobacteria as a test for oxygen in concentrations less than 0.001 per cent was used in many of the experiments.

The recording surface chosen was that of smoked paper on a revolving kymographion. The long drawn-out beat, the systole alone covering sometimes 20 seconds, permits of satisfactory recording on such a surface.

The galvanometer used was a Moll whose whole period, with magnetic shunt set at lowest position, is about 6.5 seconds and sensitivity about $5 \cdot 10^{-8}$ volt per millimeter deflection at scale distance of 132 cm. The internal resistance of the galvanometer is about 62 ohms, the external resistance varied with the thermopile used, that is, between 14 and 15 ohms. The movement of the galvanometer spot was recorded by the observer. This is made possible by a dial-hand mounted on a delicate carriage that can be rolled along in front of the galvanometer scale. The carriage is attached by cable (which moves over a pulley at right angles) to one end of a writing stylus that is operated vertically between guide-spots. The excursions of the galvanometer-spot on scale thus can be quite satisfactorily followed and recorded on the drum surface. The method was described briefly in an earlier paper (1922). Synchronously with the myogram and the thermocardiogram are recorded automatically a time-trace of uniform time intervals.

The muscle-lever is connected with the muscle by means of a light, rigid rod that passes vertically through a minute opening of a glass tube in turn passing through the top of the muscle chamber, the tube being provided with an oil-trap and bit of rubber tubing. When changing solutions or gas the rubber tubing is clamped tightly shut, around the connecting rod. When the muscle movement is to be recorded, the clamp is released, and the oil in the trap prevents diffusion of gases while the connecting rod is free to move.

2. In order to analyze the compound waves of the *thermocardiograms*, as the graphic records of these heat waves occurring during the heart-beat were designated (1922), heretofore I have used the method that already

had been described and employed by Hill and Hartree (1920) for the analysis of the waves of heat production in skeletal muscle. These authors regarded the recovery-wave as a compound sound-wave and accordingly analyzed it into its constituent elementary waves, the quantity of heat being put equal to the integral of the elementary waves. This method will be referred to hereafter for the sake of brevity as the S. W. A. (sound-wave-analysis) method. In practice the method is a tedious one, and in casting about for an easier, and if possible a surer, method it occurred to me that under proper conditions the *area* of the galvanometer's deflection to a thermo-electric current when recorded graphically on a moving surface ought itself to be a reliable measure of the total energy required to give the deflection. All that would be necessary is to complete the perimeter of the recorded deflection by projecting the null points of the galvanometer before and after the deflection as a base-line. In practice this means for thermomyograms the determination of the level of the muscle's resting heat production. In this study the points I have taken for this base-line are those representing the "coldest" points in two successive records. Often, as a matter of fact, the base line was a nearly straight line (perpendicular to the direction of galvanometer deflection) for many successive beats, a fact which indicates an excellent condition of temperature equilibrium having obtained at the time within the muscle-chamber.

There are two well-known methods of measuring areas of irregularly shaped surfaces; one involves the use of a carefully calibrated and reliable planimeter, the other consists of cutting out the areas directly (or after retracing them on paper of even thickness) and then weighing them. In this investigation the planimeter method was used.

The advantages of the area method of measuring thermomyograms over the more laborious S. W. A. method are obvious. Only, certain conditions must be observed. 1. The galvanometer deflections should vary linear within the limits of the variations of the electric current it has to record. 2. The recording surface should move at a uniform rate throughout each thermomyogram, and also preferably throughout all the observations of an experiment. In any event a time-trace should be recorded synchronously with the tracing of the galvanometer deflections. If then from time to time the speed of the recording surface varies, the areas of the galvanometer deflection can be reduced to a selected standard speed of movement. In the present work the standard speed chosen, to which all observed speeds here reported have been reduced, is 10 mm. per minute. 3. The construction of the thermopiles and the method of applying them to the muscles, the size and thickness of the muscles (factors that determine the rate of cooling), and the period of the galvanometer, as compared with the duration of the muscle's contraction, are all factors that must be so chosen and controlled that there can be no doubt but that *a*, if any, only a negligible

amount of heat is conducted or lost by convection from the warm to the cold junctions while the temperature change of the muscle is being recorded; *b*, the temperature measuring instruments (thermopile and galvanometer) register the temperature change completely or nearly completely before any appreciable amount of heat is lost to the exterior of the muscle.[1]

If the above conditions are fulfilled it becomes clear that the areas described by the galvanometer's deflection on a moving surface must be an integration of the temperature changes of the muscle, and therefore a graphic representation of the net energy that has been degraded. Finally, by recording the galvanometer's deflections to known quantities of electric current, the areas of deflection can be calibrated whereupon one can put unit area of heat-wave equal to unit quantity of work-done or calories of heat liberated.[2]

In the present paper the observed areas of the thermocardiograms are dealt with directly without attempting to convert them into absolute equivalents of energy. They are reduced, however, to a common unit of area corresponding with a surface moving at a uniform rate of 10 mm. per minute and a heart-beat of 60 seconds' duration, and then divided through by the maximum total tension developed by the heart muscle during the beat. The calculation is expressed by the ratio, $\frac{600\theta}{D_r C_c T_t}$, θ being the observed area in terms of mm.^{2}, D_r the observed rate of movement of the recording surface per minute, C_c the observed period of the complete cardiac cycle in seconds, and T_t the observed maximum total tension in grams. The area observed may be for the total period of heart beat, when it is put as θ_t; or for the phase covering systole and the active part of diastole, when the area is designated as θ_{sd}; or for the period when there is no tension change recorded by the muscle, that is for the period of the heart's pause, when it is designated as θ_p. These two chief waves of the thermocardiograms of slowly beating hearts, as described above, can often be readily delimited and their areas measured separately. In all cases the *whole* area of any thermocardiogram can be measured.

The planimeter used in the present work is figured as no. 3 on page 92 of Wheatley's book on *The Polar Planimeter* as published by Keufel and Esser Co., New York, 1903. Testing the instrument against the most accurately "engine-ruled" paper obtainable in the market and choosing areas of variously sided parallelograms, I found readings as follows:

[1] This problem in all its detail has been fully discussed with my colleague, Prof. Albert H. Pfund of the Physics Department of this University. I wish to take this occasion to thank Professor Pfund for the many times he has given me helpful advice and criticism.

[2] This method also was discussed with Professor Pfund who concurred fully with me in the correctness of the principles involved.

Area, as engine-ruled.....1.0, 1.0, 1.0, 2.0, 4.0, 4.0, 6.0, 6.0, 9.0, 25.0
Area, as measured by planimeter.....1.0, 1.1, 1.1, 2.0, 4.2, 3.9, 6.0, 6.0, 9.1, 25.3

It was concluded that the accuracy of the planimeter was in most cases better, than that of other parts of the instrumentarium of the experiments.

4. The data and results of the analyses of the thermocardiograms by the

TABLE 1

Data from the analyses of graphic records of beating ventricle, at ±0.0°C.

REMARKS	TIME	GAS USED	SERIAL HEAT NUMBERS ANALYZED	DRUM RATE (D_r)	CARDIAC CYCLE (C_c)	TENSION MAX. TOTAL (T_t)	HEAT PRODUCTION DURING: Syst. diast. (θ_{sd}) area		Pause (θ_p) area		Total beat (θ_t) area	
	hr.,min.			*mm. per min.*	*secs.*	*gm.*	*mm².*		*mm².*		*mm².*	
Exp't. of ix/29/1932.	1:05	N_2 begins										
	1:57	Nitrogen	21									
Muscle wt., 1.13 gm.		Nitrogen	22									
		Nitrogen	23	29.2	60	19.6	86		31		117	
		Nitrogen	24									
	2:02	Nitrogen	25									
	3:09	Nitrogen	29				87		50		137	
		Nitrogen	30				100		33		133	
		Nitrogen	31	27.9	65	22.5	83		24		107	
		Nitrogen	32				73		44		117	
	3:15	Nitrogen	33				90	86	27	37	117	122
	4:12	Nitrogen	41				73		87		160	
		Nitrogen	42				77		43		120	
		Nitrogen	43	23.1	69	21.4	90		40		130	
		Nitrogen	44				77		60		137	
	4:17	Nitrogen	45				87	81	55	57	143	138
		O_2 begins										
	4:39	Oxygen	49				67		63		130	
		Oxygen	50				67		63		130	
		Oxygen	51	22.7	61	27.4	73		40		113	
		Oxygen	52				67		66		133	
	4:45	Oxygen	53				80	70	73	62	153	132
	5:15	Oxygen	61				67		36		103	
		Oxygen	62				80		50		130	
		Oxygen	63	18.7	52	24.4	67		40		107	
		Oxygen	64				83		37		120	
	5:20	Oxygen	65				67	73	50	42	117	115

TABLE 1—*Concluded*

REMARKS	TIME	GAS USED	SERIAL BEAT NUMBERS ANALYZED	DRUM RATE (D_r)	CARDIAC CYCLE (C_c)	TENSION MAX. TOTAL (T_t)	HEAT PRODUCTION DURING					
							Syst. diast. (θ_{sd}) area		Pause (θ_p) area		Total beat (θ_t) area	
	hr.,min.			*mm. per min.*	*secs.*	*gm.*	*mm².*		*mm².*		*mm².*	
Exp't. of xii/30/1932. Muscle wt., 1.61 gm.	12:37	N_2 begins	8				110		80		190	
		Nitrogen	9				110		100		210	
		Nitrogen	10				107		100		207	
		Nitrogen	11	42.1	67	69.2	117		80		197	
		Nitrogen	12				100		100		200	
		Nitrogen	13				90		107		197	
	12:53	Nitrogen	14				117	109	90	93	207	202
	2:28	Nitrogen	28				80		70		150	
		Nitrogen	29				70		60		130	
		Nitrogen	30	24.1	86	30.9	70		75		145	
		Nitrogen	31				80		70		150	
	3:14	Nitrogen	32				70	74	70	69	140	143
	3:57	O_2 begins										
	4:04	Oxygen	37				170		65		225	
		Oxygen	38			29.3	110		77		187	
		Oxygen	39	42.6	66		140		65		205	
		Oxygen	42				143		70		213	
	4:20	Oxygen	43			25.3	124	137	55	64	179	202

area method are illustrated in the tables below. Tables 1 and 2 contain detailed observations on two pairs of auricles and three split ventricles. They show the variations in area of heat waves (θ) that are liable to occur 1, when the tension development (T_t) with each beat remains fairly constant and also when it varies; 2, when the duration of the beat (C_c) varies; 3, when the rate of movement of the recording surface (D_r) varies, and 4, when the gas supply to the muscle is changed from oxygen-plenty to oxygen-lack. The "serial beat numbers," put in a separate column, refer to the beats as they occur in succession on the records. Those beat numbers not appearing in the tables are either those whose records were marred by some defect in technique, or those that are mere repetitions of the ones tabulated. The right-hand digits of all the figures in the tables are only approximately correct. The observed data under the headings D_r, C_c and T_t thus are the characteristics of any one beat, which are finally simplified into a single letter, gamma, and used as denominator of the ratios appearing in the last three columns of table 3. These ratios express the reduced areas for total beat (θ_t), the systole-diastole portion of the beat

(θ_{sd}) and for the period of pause (θ_p) respectively. For the calculation of these areas, as will be noted, only the mean values of the variable, observed factors for groups of several beats are taken. The calculation thus reduces the observations to areas corresponding to the fixed and standard conditions of a total tension development of one gram per beat lasting 60 seconds, recorded on a surface moving at uniform rate of 10 mm. per minute. In comparing these areas the reader should keep in mind that, while they have

TABLE 2

Data from the analyses of the graphic records of beating auricles

REMARKS	TIME	TEMP.	GAS USED	BACT. GLOWING	SERIAL NUMBER OF BEAT	D_r	C_r	T_l	θ_{sd} AREA	θ_p AREA	θ_l AREA
	hr., min.	*°C.*				*mm. per min.*	*secs./ beat*	*gm.*	*mm².*	*mm².*	*mm².*
Exp't v/16/31.	12:30	−0.4	Oxygen	+	6	19.6	73	10.2	420	240	660
Wt. of muscle, 0.52 gm.			Oxygen	+	7	19.6	137	10.2	420	830	1250
			Oxygen	+	8	19.6	62	10.4	420	210	630
	12:55		Oxygen	+	9	19.6	62	10.2	350	200	550
	2:20	+0.5	N_2 begun:								
	3:56		Nitrogen	0	22–26	20.8	62	6.3	154	176	330
	4:30		Nitrogen	0	31–33	42.3	59	5.5	420	280	720
	4:45		Nitrogen	0	42–44	34.7	61	7.7	413	243	656
	4:57	+1.0	Nitrogen	0	45–48	10.0	64	6.9	132	62	194
Exp't v/3/32.	5:00	+0.2	N_2 begun:								
Wt. of muscle, 0.60 gm.	6:20	+0.2	Nitrogen	+	5–6	11.5	283	11.4	33	130	163
	6:54	+0.3	Nitrogen	+	7–8	7.9	282	10.6	52	63	115
Cont'd. v/4	11:40	+3.0	Oxygen	+	37–40	16.6	240	7.7	29	123	152
	12:00		N_2 begun:								
	4:20	+5.0	Nitrogen	0	54–56	17.0	137	5.6	170	62	232
	4:30	+5.0	Nitrogen	0	57–59	17.0	117	5.4	119	93	212
	5:04	+5.0	Nitrogen	0	71–75	13.2	113	8.4	72	69	141

no significance in terms of absolute heat units, they have a reliable relative value one with another all within any one experiment; and as long as thermels[3] and galvanometer characteristics remain the same, the areas of different experiments so reduced are also comparable. However the thermels and galvanometer used in the older experiments were not the same

[3] Professor Pfund has suggested that *thermel* be used as a substitute for the longer term "thermoelectric element." *Thermels* would seem to be the logical term for a chain of the elements.

TABLE 3

*Heat production of heart muscle reduced to standard conditions**

EXP'T, DATE, AND MUSCLE USED	GAS USED	SERIAL NUMBERS OF BEATS	D_r	C_c	T_t	θ_t	θ_{sd}	$\frac{\theta_t}{\gamma}$	$\frac{\theta_{sd}}{\gamma}$	$\frac{\theta_p}{\gamma}$
ix/29/32	*Nitrogen:*	21–25	29	60	19.6	117	86	(2.05)	(1.51)	(0.43)
Ventricle	(no bact.	29–33	28	65	22.5	122	86	1.78	1.25	0.53
	test)	41–45	23	69	21.5	138	81	2.43	1.42	1.01
		Mean,						**2.10**	**1.33**	**0.77**
	Oxygen:	49–53	23	61	27.4	132	70	2.07	1.11	0.96
		61–65	19	52	24.4	115	73	2.92	1.86	1.06
		Mean,						**2.50**	**1.49**	**1.01**
xii/30/32	*Nitrogen:*	8–14	42	67	69.2	202	109	**0.61**	**0.33**	**0.28**
Ventricle	(no bact.	28–32	24	86	30.9	143	74	1.34	0.69	0.65
	test)									
	Oxygen:	37–39	43	66	(29.3	202	137	**1.56**	**1.01**	**0.55**
		42–43			25.3)					
v/20/27	$CN + N_2$:	11–13	25	68	34.0	149	107	1.55	1.11	0.44
Ventricle		14–17	25	68	34.0	161	111	1.67	1.15	0.52
		Mean,						**1.62**	**1.13**	**0.49**
	Oxygen:	42–44	21	64	60.0	304	178	2.26	1.32	0.94
		45–47	35	62	61.0	537	351	2.47	1.59	0.88
		48–49	33	62	61.0	526	334	2.53	1.61	0.92
		Mean,						**2.40**	**1.49**	**0.91**
v/16/31	*Oxygen:*	6	20	73	10.2	660	420	2.40	1.52	0.88
Auricles		7	20	137	10.2	1250	420	2.73	0.92	1.81
		8	20	62	10.4	630	420	3.01	2.01	1.00
		9	20	62	10.2	550	350	2.68	1.71	0.97
		Mean,						**2.71**	**1.54**	**1.17**
	Nitrogen:	22–26	21	62	6.3	330	154	2.44	1.14	1.30
	bacteria do	31–33	42	59	5.5	720	420	3.13	1.84	1.33
	not glow	42–44	35	61	7.7	656	413	2.42	1.52	0.90
		45–48	10	64	6.9	194	132	**2.63**	**1.79**	**0.84**
v/3/32	*Nitrogen:*	5–6	12	283	11.4	163	33	2.53	0.51	2.02
Auricles	bact. are	7–8	8	282	10.6	115	52	2.88	1.31	1.57
	glowing									
	Oxygen:	37–40	17	240	7.7	152	29	**2.98**	**0.57**	**2.41**
	Nitrogen:	54–56	17	137	5.6	232	170	1.10	0.70	0.40
	bact. do	57–59	17	117	5.4	212	119			
	not glow	71–75	13	113	8.4	141	72	**0.67**	**0.34**	**0.33**

* The factor γ in the ratios in the captions of the last three columns is constant for each beat or group of beats and is equal to $600/D_rC_cT_t$.

as in those of the newer, and no correction has been made for the differences of their characteristics. The magnitudes of the figures from one experiment therefore should not be compared with those of another, although since the characteristics of galvanometers and thermels do not vary so much these magnitudes also do not so greatly differ.

5. It would be of interest to compare figures from thermocardiograms that have been measured by both the "area" and the "S. W. A." method. The readings by the S. W. A. method are reduced to standard tension but not to standard rate of beat. The method itself, if properly applied automatically corrects for any differences in rate of movement of recording surface; the readings therefore require no correction for that factor. In order to put the readings of the two methods in figures as nearly comparable as possible, therefore, those of the area method are reduced only to standard drum rate and standard tension by the ratio $10\ \theta/D_r T_t$. Since the results of the two methods are stated in different denominations they cannot be compared figure for figure but certain relations may be compared such as the percentage gain in heat production upon readmitting the muscle from a condition of oxygen-want to one of oxygen-plenty. In view of this limitation no extensive comparison of the results of the two methods at this time is warranted. I therefore take only a few thermocardiograms, from the experiment of v/20/27, which have already been dealt with above by the area-method. The table below puts the results in parallel:

SERIAL NUMBERS OF BEATS	OXYGEN SUPPLY	AREA-METHOD, HEAT PRODUCTION IN UNITS OF MM².		S.W.A. METHOD, HEAT PRODUCTION IN TERMS OF GM—CAL	
		S-diastole	Pause	S-diastole	Pause
11–17	O_2-want	1.10	0.33	16.6	2.0
45–49	O_2-plenty	1.57	0.88	15.7	6.2
Per cent gain in O_2-plenty...		42.7	166	−5.4	210

Both methods agree in showing *a*, not only an increased rate of heat production by the muscle upon readmission to oxygen but total percentage gains that are of almost equal magnitude, that is, a total gain of 42.7 + 166, or 208.7 per cent, by the area method as against −5.4 + 210, or 204.6 per cent, by the S. W. A. method, but also *b*, an unusually low amount of heat production during the period of pause (recovery phase) when compared with the period of systole-diastole.

The two methods differ very much however in the way they distribute the gains in heat production between the two phases of the heart cycle. The area method registers a marked gain in heat production during the systole-diastole (initial) phase whereas the S. W. A. method registers no gain at all for this phase. On the other hand, the gain registered by the

area method for the period of pause (recovery) is considerably less than the gain registered by the S. W. A. method. This failure of the latter method to show increased heat production in oxygen-plenty over that in oxygen-want during the *sd*-period, however, is exceptional rather than the rule, as one will see by referring to my paper of 1928 where the S. W. A. method was used for the measurements.

It should also be pointed out that the heat production in oxygen-want measured by the area method is not always less than the heat production in oxygen-plenty as will be noted in the experiment of v/16/31 as summarized in table 4. It should be remembered, however, that the measure for "initial heat production" by the S. W. A. method has been the linear vertical deflection of the galvanometer on scale. That is, it has been regarded in practice as an elementary wave, requiring no further analysis. For long drawn-out contractions, such as cardiac muscle undergoes at zero

TABLE 4

Percentage gain in heat production in oxygen-plenty over the heat production in oxygen-lack, based on data in table 3, using the figures in heavy-face type only

KIND OF MUSCLE, AND DATE OF EXPERIMENT	DURING SYSTOLE-DIASTOLE	DURING THE PAUSE	DURING THE WHOLE BEAT
Auricle, v/16/31	−16	25	3
Auricle, v/3/32	68	631	344
Ventricle, ix/29/32	20	133	19
Ventricle, xii/30/32	206	96	156
Ventricle, v/20/27	32	65	48

degrees, the height of galvanometer excursion may well become inadequate as a measure of total energy for the heat production in the first wave.

6. A comparison of the results obtained by my area method shows a number of positive results, some of which are corroborative of earlier findings. In all there are 28 successful experiments on heart muscles alone upon which the general statements that follow are based.

a. Even after reduction to standard units of tension development and duration of beat the amounts of heat production both in auricular and ventricular muscle at times and in successive beats show considerable variation.

b. Although in well-aereated preparations the total heat production occasionally may be no more, and sometimes may be even a little less, than in preparations deprived of oxygen, nevertheless in general the total heat production per beat is much greater in oxygen-plenty than in oxygen-want. The findings are illustrated in table 4, and agree with those of the paper of 1927. The gains in greatest heat production in O_2-plenty over the least heat production in O_2-want for the whole period of the beat are 3 per cent and 344 per cent for the two auricles, 19, 48 and 156 for the three ventricles.

Analyses of total heat production of three other ventricles of the 1932 series and not included in the table show mean gains of 34, 31 and 20 per cent. In view of this wide variation no total mean can be stated for the gain in O_2-plenty over the heat production in O_2-want.

c. The heat liberated during O_2-plenty shows a gain over the heat production in O_2-want not only, as one would expect, during the phase of quiescence following tension-change but also during the phase of tension-change itself. While it is not true for all beats that the magnitude of the gain varies widely, nevertheless there is a tendency for the gain to be greater for the period of quiescence than for the period of tension-change (systole-diastole). See table 4.

d. The heat production during diastasis, as one would expect, is to some extent a function of time, and if the period of diastasis varies in successive beats then θ_p also varies. This point is illustrated in the experiment of v/16/31. See table 3. The auricles, having suffered momentary partial block,

TABLE 5

BEAT NUMBERS	AREAS OF HEAT PRODUCTION OBSERVED:			AREAS OF HEAT PRODUCTION REDUCED TO STANDARD CONDITION			
	θ_{sd}	θ_p	θ_t	$\frac{200\,\theta_{sd}}{D_r S d_p}$ (1)	$\frac{400\,\theta_p}{D_r P_p T_t}$ (2)	Sum of (1) and (2)	$\frac{600\,\theta_t}{D_r C_c T_t}$
6	420	240	660	1.96	0.91	2.87	2.40
7	420	830	1250	2.06	1.39	3.45	2.73
8	420	210	630	1.83	1.02	2.88	2.98
9	350	200	550	1.56	0.97	2.62	2.66
Dev....	±35	±315	±350	±0.25	±0.24	±0.41	±0.29

or some defect of conduction, beat-numbers 6, 7, 8 and 9 show markedly variable values for observed θ_p. The original records show that drum rate, tension developed and period of systole-diastole all remain fairly constant, but the period of pause (P_p) alone varies, being 52, 117, 40 and 40 seconds, corresponding to the observed values of θ_p, 240, 830, 210 and 200, respectively. If one reduce the observed values of θ_p not only to the standard drum rate and tension but also further to a standard period of diastasis of 40 seconds, by the ratio $400\,\theta_p/D_r P_p$, then we have values of heat production whose deviation (of extremes from the mean) is reduced from ±315 to ±0.24 (see table 5).

e. As already stated the durations of systole-diastolic phase, in the four beats just considered, are fairly constant in the records, likewise their corresponding heat production in terms of area (θ_{sd}). Reducing to a standard heat production of constant systole-diastolic period (say 20 seconds) therefore would add nothing to the uniformity of the $\theta_{sd}/D_r T_t$

ratio, and correcting to a uniform beat of 60 seconds worsens it distinctly, as shown under the θ_{sd}/γ ratios in table 3. In general, however, if reduction is made at all to some standard systole-diastole duration, for this heart it should be to a 20-seconds period; the ratio for the calculation would be $200\,\theta_{sd}/D_rSd_pT_t$, where Sd_p is the observed time of systole-diastole tension-change.

In table 5 the observed areas of heat production of the four beats just considered are put down along with their values after reducing by the ratios suggested above as appropriate. The extreme deviation from the mean (of the extremes) is appended below each column. It will be noted in this table that the sums of the corresponding figures in columns (1) and (2) do not equal the corresponding figures in the last column, as we would expect them to do. The reason for this must be that errors of the two readings are additive in the one case and neutralize each other in the other case. Be that as it may, when one compares the great deviation among the observed areas for θ_p and θ_t with the small deviations after reducing to standard conditions the importance of the procedure becomes evident.

f. So far as smooth muscle (see papers of 1927, 1930) and cardiac muscle (see paper of 1928, and the experiments here reported) are concerned it seems clear that the period of tension-change is not necessarily one of purely anoxidative changes, nor is the period of rest immediately following tension-change one of purely oxidative changes. For we find usually both a marked heat production in both these phases after oxygen has been removed to the utmost, and an increased heat production in both phases during oxygen-plenty over and above that liberated in oxygen-want.

While continuance of initial heat production in oxygen-want is evidence in favor of the view that the energy for contraction is non-oxidative in its immediate origin, nevertheless we must admit that the observed heat production is only a net remainder of energy exchanges about which we know even today all too little. The gain in heat production during tension change of smooth and heart muscles in O_2-plenty most probably is associated directly with the processes preparatory to, rather than directly with, the contractile process itself. On the other hand the recovery processes most probably go on bit by bit, regardless of the acts of contraction, normally salvaging the products of breakdown, no doubt at greater rate when the latter are accumulating at greater rate, but in no wise necessarily step for step with the breakdowns of tension changes. The heat productions of the heart's beats in oxygen-plenty, therefore, may well be anything but uniform in quantity, although the rhythmical processes of tension production and tension release may facilitate greatly and accelerate all the recovery processes. The physical and chemical changes among the materials and conditions coincident and requisite for the oxidations and recovery processes themselves must likewise vary from time to time

even from beat to beat in kaleidoscopic fashion. This view seems all the more probable when we remember that the processes of recovery are not all oxidative and that those that are oxidative are not to be viewed as coupled reactions of those that are anoxidative (Bancroft and Bancroft, 1930; Amandus Hahn, 1931).

g. It appears, therefore, no longer a tenable inference that the net heat given off in absence of oxygen during tension-change in smooth or cardiac muscle is all, or even in large part, necessarily directly concerned with the energy required to produce that tension-change. Some small part of the heat however surely must be a constant net remainder of the energy released. The part liberated over and above, having no direct concern with this obligate minimum whatever, may be of marked variety in magnitude. And so it is in fact, although less varied than the heat production following hard on the contractile act.

But do we ever make a reading of an initial heat production that is the mere obligate minimum of the contractile act itself? If so, what is its magnitude and under what conditions do we observe it? For skeletal muscle the answer is not yet complete; for smooth and cardiac muscle it is far from final. But certain features of changes in quantities of heat production have occurred in a number of my experiments that seem to point the direction in which the answer lies.

Upon removal of oxygen to the utmost, the diminution of heat production appears first in the recovery phase as was originally observed by Hartree and Hill for skeletal muscle. But as the time in oxygen-lack is prolonged, a very marked diminution of heat production also may appear in the initial phase of the muscle action. This marked diminution I do not believe will be observed, unless the oxygen-withdrawal has reached the point where photobacteria cease to glow; it does not always occur even then. But once having dropped to the minimum level readmission of the oxygen to the muscle-chamber, sufficient to allow the photobacteria to glow, usually will show an increase in initial heat production of the muscle, be it smooth or cardiac.

In the 1928 study on heart muscle the table on p. 77 summarizes the observations in heat production of five ventricles, two of which show the systolic heat production in oxygen-lack to be about $\frac{1}{3}$, two others show it to be about $\frac{1}{2}$ of what it is in oxygen plenty; the only heart out of the five showing moderate reduction is the one that was exposed to oxygen-lack the shortest time. Of the five heart preparations reported in the present (1932) series three show their lowest systolic heat production in oxygen-want to be about $\frac{2}{3}$ of the highest observed in oxygen-plenty; another shows a ratio of $\frac{5}{8}$, but the ventricle of date xii/30/32, shows again that systolic heat production in oxygen-want may be as low as only $\frac{1}{3}$ of what it may be in oxygen-plenty.

Those experiments on smooth-muscle, summarized on p. 124 of the 1930 study, show that the minimal initial heat production of that muscle in oxygen-want may be as low as $\frac{1}{4}$ of the values in oxygen-plenty. In this latter study, the observations having been reduced to absolute heat units, a comparison of the energy liberated with the probable chemical changes could be made. The comparison indicated that anoxidative heat production represented a quantity of energy nearly equivalent to the amount Meyerhof and Schultz had found to be liberated in the breakdown of carbohydrate to lactic acid when one gram skeletal muscle in the twitch exerts a maximum tension of one gram. It now becomes probable that phosphogen rather than lactic acid is concerned; but it has also been shown that the heat production in phosphogen breakdown is the same as in lactic acid breakdown (Meyerhof, Lundsgaard and Blascho). In the case of smooth muscle thus there was no excess of anoxidative heat production left over to explain. The occasionally low ratios of anoxidative heat production to oxidative heat production, during the contractile act that continue to be found for heart muscle suggests that under certain conditions cardiac muscle also will fail to liberate more heat during the initial phase of contraction than the net amount furnished by the minimal chemical reactions required for the tension change. The heats of other sources in these cases then must be of such magnitudes and signs as to neutralize each other during the systole-diastolic period. But what the conditions are that cause these variations one cannot now say. One may only surmise that the enzymes and catalysts, controlling anaerobic break-down and oxidations, themselves vary greatly in their activities, which in turn cause variations in the observed heat production.

h. What part of the heat observed during the beat is due to oxidative chemical reactions? The observations during anaerobiosis vary greatly, from which we infer that the amount of fermentative breakdown also varies greatly. If this is the case in the absence of oxygen may it not then also be the case in the presence of oxygen? If so, then to subtract the heat observed in O_2-want from that observed in O_2-plenty and say that the difference is due directly to oxidative chemical reactions is not a valid conclusion; for it is obvious that the anaerobic breakdowns at any time may be more or may be less. Only when and if the chemical exchanges of each beat are measured along with the heat exchanges then may we be fully justified in comparing them. Judging from the heat production for single beats it seems probable that the anaerobic reactions have a tendency to subside to the minimal obligate quantities in the presence of excess of free oxygen. But the finding that the amount of heat usually exceeds the probable net requirement for the tension change produced seems rather to indicate that the heart tissues (at least under experimental conditions) are prodigal in the chemical reactions concerned with the work they have to do.

An explanation of this prodigality may be had if we imagine that these chemical reactions are not confined to the *Kontraktionsorte* of Meyerhof, but that they take place also outside these points as well, where the energy they yield is lost so far as tension change is concerned. This would be somewhat analogous to the case of the stream that feeds a millrace; all the water finally gets down to the lower level of the water-wheel, but only that part of the stream which is led successfully into the tube feeding the turbines is actually turned into effective energy, the rest of the energy of the stream is lost or diverted. This latter part may be a very variable quantity, what with drought and freshets. And so also must it be with the fount of energy supplying muscle, if we are to believe our observations. Only the part of the reaction that takes place within the essential contractile elements, so long as the amount remains constant, leads to constant tension change. The great variations both in heat production and in chemical exchanges observed to occur especially in smooth and cardiac muscle then may well be due to those exchanges going on outside the contractile elements of the muscle fibers which have nothing to do directly with their mechanical responses. In the light of this view to expect the total chemical changes to bear any exact relations to the single mechanical responses of the muscle therefore becomes a purely gratuitous assumption.

SUMMARY

A new method is described for the analysis and measurement of waves of heat-production as recorded in thermomyograms. Analyses of specimen records of terrapin ventricle and auricles under conditions of oxygen-plenty and oxygen-want are reported. Comparison of the results indicate a statistical tendency to increased heat-production in oxygen-plenty over that found in oxygen-want not only during the period of the beat following tension change but also during the period of tension change itself. The individual hearts and from time to time the same heart however shows great variation in heat production. Considering the probable chemical exchanges involved both in oxygen-plenty and in oxygen-want it is suggested 1, that the amounts and kinds of anaerobic chemical reactions are probably rarely the minimal and obligate reactions required for the actual tension change set up, but rather are usually in great excess in quantity, and also in kinds of what is immediately required for the tension developed; 2, that the oxidative reactions in presence of oxygen likewise probably are of very variable quantities during the beat, and that since they proceed *pari passu* with variable anaerobic reactions it is impossible to state what share of the total heat during a beat may be regarded as the net heat production of oxidative origin. To account for these variations usually in excess of the probable requirement, it is suggested that those chemical reactions concerned with tension change in smooth and cardiac muscle, at

least under experimental conditions, probably take place also outside the contractile elements in the fibers in variable amounts.

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THE DECREMENT IN MUSCULAR FORCE WITH INCREASING SPEED OF SHORTENING

H. C. STEVENS AND R. P. METCALF[1]

From the Psychological Laboratory of Oberlin College, Oberlin, Ohio

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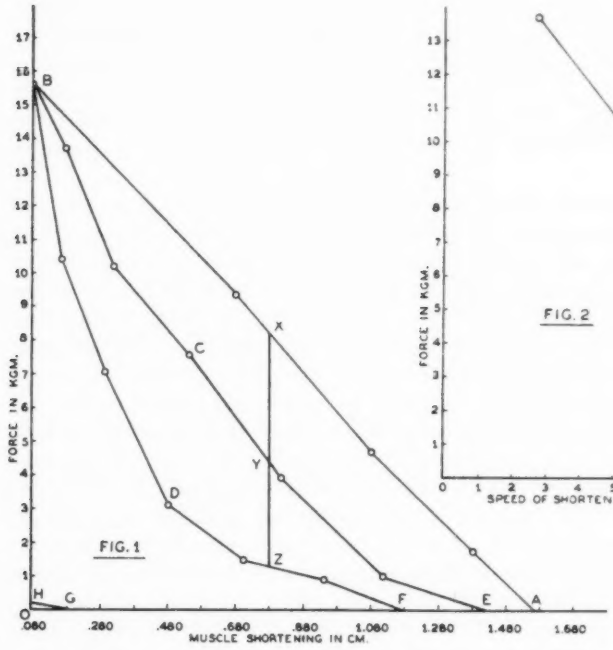
One of the outstanding facts of muscle physiology is the sudden and marked decrease in force which occurs when a contracting muscle is allowed to shorten continuously. This fact can be demonstrated by determining the stress-strain diagram of the same muscle first under isometric and immediately afterwards under isotonic conditions. Such an experiment (C520) is shown in figure 1. The area *AOB* is the isometric tension-length area. This area is determined by measuring the force of the muscle at a fixed, initial length when stimulated by a maximal tetanic, stimulus. The two ends of the muscle are then approximated by a measured distance. The muscle is again stimulated; it contracts isometrically at the new length and the force is measured as before. This procedure is repeated by gradations of 0.3 cm. until the initial length of the muscle, stretched by a force of 200 grams, has been diminished by 1.3 cm. When the forces measured in such an experiment are plotted against the distances by which the initial length is diminished, there results a triangular area like *AOB* of figure 1. This area which has the dimensions of work, namely, force and distance, has been called by A. V. Hill (1) the theoretical maximum work to distinguish it from the work obtained when the muscle actually shortens. While the area *AOB* relates force to distance, it should be understood that it does not represent work done by the muscle for the reason that the muscle does not shorten when the forces measured are developed.

The work done when the muscle shortens against an inertia of an equivalent mass of 137.6 kgm., is represented by the area *EGHB*. The loss in force or the force decrement due to shortening is shown strikingly by the vertical distance between the lines *AXB* and *ECB*. It should be noted that the force exerted is indicated by the ordinates of the lines *AXB* and *ECB* at the same length of muscle. Thus, for a shortening of 0.78 cm. (indicated by the line *XYZ*, fig. 1) the isometric force is 8.2 kgm., while in contracting against an equivalent mass of 137.6 kgm., the isotonic force

[1] Read at the Cincinnati meeting of the Federated Societies of Experimental Biology and Medicine, April 10, 1933.

is 4.2 kgm. With an equivalent mass of 12.8 kgm., the force exerted was 1.3 kgm. The only difference in the mechanical condition of the muscle in the three experiments is that, in the isometric condition, the muscle shortened to a certain, fixed length and was not allowed to shorten more. In the isotonic condition the muscle shortened continuously as far as it



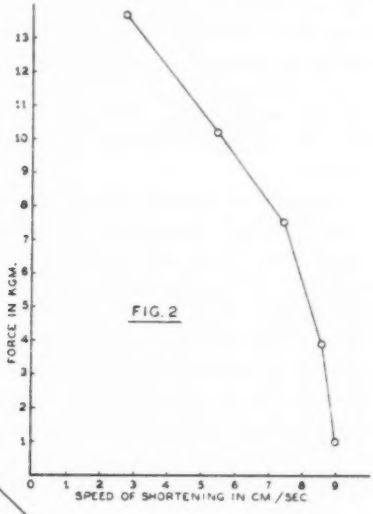


Fig. 1. Tension length diagrams with isometric technique and with isotonic technique for two equivalent masses. *AOB* is the isometric tension length area representing the theoretical maximum work. *EGHB* is the isotonic tension length area with an equivalent mass of 137.6 kgm. *FGHB* is the isotonic tension length area with an equivalent mass of 12.8 kgm. The vertical distance between the isometric and the isotonic curves represents the force decrement due to increasing speed of shortening at the same length of muscle.

Fig. 2. This graph relates the force developed to speed of shortening with the muscle contracting against an equivalent mass of 137.6 kgm.

could and with increasing speed. With the isometric technique, the length at which the muscle exerts its force is fixed for the duration of the contraction which is about 200 sigma: while with the isotonic technique, the length is an instantaneous length of a continuously shortening muscle. It is evident, therefore, that some force-reducing factor of a very considerable

magnitude is at work when the muscle shortens continuously. It is the purpose of these experiments to show how this force-reducing factor behaves with respect to increasing speed of shortening.

A quantitative study of the change of length of the muscle together with its speed of shortening and force exerted shows that the maximal force of the muscle is exerted at a length only slightly less (0.177 cm.) than the initial length of the muscle. The force is not constant. It falls off with time in an approximately linear fashion. In some animals the force-time curve is somewhat concave upwards especially at the lower end. The speed of shortening at first increases approximately uniformly then rounds off and finally falls. Force decreases as speed of shortening increases (fig. 2). The data from which these curves may be plotted are to be found in table 1.

A study of figure 1 shows, however, that the loss of force is due not merely to the shortening of the muscle but is also dependent upon the speed with which the shortening is carried out. In this experiment the speed of shortening was varied by changing the equivalent mass of the inertia disc against which the muscle contracts. In figure 1, the area *EGHB* represents the work done by the muscle in 200 sigma in shortening against an equivalent mass of 137.6 kgm. The area *FGHB* is the work done by the same muscle in 67 sigma when contracting against an equivalent mass of 12.8 kgm. The muscle was at work three times as long in the first instance as in the second and the work done was 100 per cent greater. The work done on the muscle by the initial tension of 200 grams is represented by the triangle *GOH* which is very small on the scale to which this diagram is drawn. Measurements made from an "instrumental run" show that work done on the muscle amounts to 8,019 ergs or 0.0081 kgm.-cm. of work. With an infinitely slow rate of shortening, the isotonic area would approach as a limit the isometric area and the realizable work would tend to approximate the theoretical maximum work. Since it is now evident that the magnitude of the force-reducing factor is dependent upon the speed of shortening, it becomes a matter of some importance to know the form of the curve which relates these two quantities.

Before presenting our results a brief description of the method and technique will be given. For a description of the apparatus and details of the method a paper by Stevens and Snodgrass (6) may be consulted. The muscle preparation used was the gastrocnemius of the decerebrate cat stimulated through the intact, tibial nerve by a rectified, 60 cycle, alternating current of 120 pulses per second. An oscillograph record of this stimulus may be found in reference (7). This current was graded by means of a voltage divider. The least maximal contraction stimulus was determined before and after each experiment. The deterioration of the muscle during the course of an experiment which ordinarily lasted about

two hours, was checked in this manner. The usual stimulus intensity was a peak voltage of 0.46 volt. The muscle which was stretched by a tension of 200 grams contracted against an inertia disc the displacement of which was recorded photographically. On the same film (fig. 5) the change of length of the muscle was likewise recorded. Time was imprinted on the record by means of a slotted disc which was rotated by a synchronous motor 30 times per second. A time interval ranging from $\frac{1}{30}$ to $\frac{1}{15}$ of a second was used for the slower and faster contractions. The speed of contraction was controlled by the equivalent mass of the inertia disc.

TABLE 1

(1) TIME	(2) MUSCLE SHORTENING	(3) MUSCLE VELOCITY	(4) ANGULAR VELOCITY OF DISC	(5) ANGULAR ACCELERATION OF DISC	(6) FORCE	(7) WORK	(8) POWER = Fv
seconds	*cm.*	*cm./sec.*	*radians/second*	*radians/second²*	*kgm.*	*kgm./cm.*	
0	0	0	0	0	0	0	0
		1.23	0.63				
0.033	0.177	(2.76)		51.6	13.70	1.26	37.8
		4.29	2.35				
0.067	0.320	(5.49)		38.4	10.20	1.86	55.9
		6.69	3.63				
0.100	0.543	(7.44)		28.4	7.54	1.87	56.0
		8.19	4.58				
0.133	0.816	(8.58)		14.7	3.90	1.11	33.4
		8.97	5.06				
0.167	1.115	(8.98)		3.8	1.0	0.30	9.0
		9.00	5.19				
0.200	1.415						

TABLE 2

FORCE	FORCE DECREMENT	MUSCLE SPEED	APPARENT VISCOSITY FORCE DECREMENT SPEED
kgm.	*kgm.*	*cm./sec.*	
13.700	1.0	2.76	0.362
10.200	2.8	5.49	0.510
7.54	3.3	7.44	0.445
3.90	3.8	8.58	0.443
1.00	3.3	8.98	0.368

The most commonly used equivalent masses were 12.8 kgm., 137.6 kgm. and 337.4 kgm. All experiments were quick releases which means that the disc was prevented from moving by means of a detent until the muscle had contracted tetanically against it. The disc was then suddenly released and the muscle allowed to shorten. The muscle was maximally stimulated by a tetanizing current both before and throughout the contraction. The duration of the stimulus is indicated in figure 5 by the blackened area at the top. This mode of stimulation was used to insure maximal force at the time of release and to prevent partial relaxation during shortening.

From the film, measurements of the displacement of the disc and of the change of length of the muscle per unit time were made by means of a

reading microscope and a Gaertner comparator reading to 0.001 cm. The reliability of the measurements is ±0.002 cm. From these measurements, the velocity of the disc, the velocity of the muscle and the acceleration of the disc were computed for each time interval of the contraction cycle for the isotonic experiment. Force, work and power were also calculated for the same time intervals. In spite of the massive metal construction of the apparatus and the initial, passive tension of 200 grams a "give" of about 0.08 cm. is measurable in our records before the detent releases. There is, therefore, an initial shortening of the muscle and a small initial velocity at the time of the release.

The muscle and nerve were kept moist with mammalian Ringer's solution. The muscle was warmed to body temperature by means of a heater made by winding nichrome wire around a glass cylinder, the temperature of the coil being controlled by a rheostat. The isometric measurements were made with a torsion wire myograph which magnified the twist of the wire 13.8 times. With this magnification, the calibration gave a displacement of 0.06 cm. for 1 kgm. of load.

While the error of the original measurements may be taken as ±0.002 cm., it was suggested that the process of double differentiation by taking the first and second differences, might greatly augment the error inherent in these readings. Since statistical methods are not applicable to this problem, a method was employed for estimating this error which was suggested by Prof. F. G. Tucker of the Department of Physics, Oberlin College. This method proceeds by three steps. First step: plot the displacement of the disc against successive, unit, time intervals. Second step: fit an empirical equation to the curve obtained in the first step. The empirical equation which was fitted to the data of C520 was:

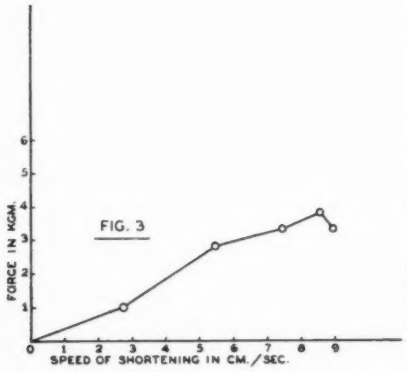
$$\text{Equation 1.} \quad Y = 114.16\,T^3 - 60.537\,T^2 - 0.8787\,T - 0.0015.$$

Third step: take the second derivative of this equation with respect to time and from the accelerations thus obtained, calculate the forces. The measured and calculated forces are shown in the following table. The difference between the calculated and measured forces will indicate the magnitude of the error due to differentiation. The maximum deviation is 6.4 per cent of the measured force.

TIME	FORCE		PER CENT OF DEVIATION
	Measured	Calculated	
	kgm.	*kgm.*	*per cent*
0.033	13.700	13.710	0.7
0.067	10.200	10.520	3.1
0.100	7.540	7.340	2.5
0.133	3.900	4.150	6.4
0.167	1.000	0.960	4.0

The results obtained from cat 520 have been chosen as typical of the fifteen animals used in this series of experiments. Altogether 112 individual records were made and measured. Of these 59 were obtained with equivalent mass 12.8 kgm.; 27 with the 137.6 kgm. and 26 with the 337.4 kgm. equivalent mass. Individual records of the same equivalent mass obtained from the same animal were averaged together. Ordinarily three or four records are combined in each result.

To determine the force decrement, the distance between the isometric and isotonic curves at the same length is measured from the tension length diagram of figure 1. These differences between the isometric and isotonic forces are taken at the length which the muscle had reached when the force



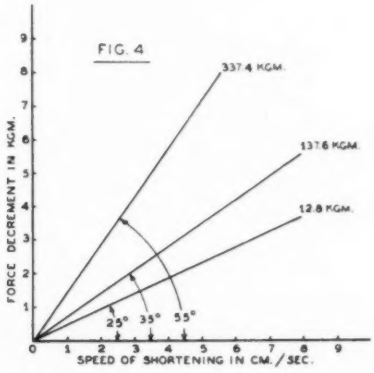


Fig. 3. This graph relates force-decrement to speed of shortening with an equivalent mass of 137.6 kgm. It shows that the loss of force is a linear function of speed of shortening except for the last part of the contraction.

Fig. 4. Graphs which show how the slope of the force decrement-speed curve increases with increase in the equivalent mass.

was measured. The speed with which the muscle is shortening when a given force is being exerted is determined from the force-speed curve, figure 2. With an equivalent mass of 137.6 kgm. the forces, force decrements and corresponding speeds of shortening were obtained which are shown in table 2.

When the force decrements of this experiment are plotted against the corresponding speeds of shortening, there results the curve shown in figure 3, the determination of which is the primary objective of this research. These curves are, with four exceptions, approximately linear; they pass through the origin of the axes and are inclined to the x-axis at a certain angle. The four exceptions are C521, C522, C533, and C535. These records have two features in common, namely, they were obtained from

TABLE 3

Distribution of slopes with reference to equivalent mass

EQUIVALENT MASS	10–19°	20–29°	30–39°	40–49°	50–59°	60–69°
kgm.						
12 8	10	24	33			
	13	21	32			
	19	25	30			
		21	33			
		23				
		24				
		25				
		20				
		23	38	45	54	
137 6			33	49		
			38			
			39		59	60
337 4			32		56	
					56	
					54	

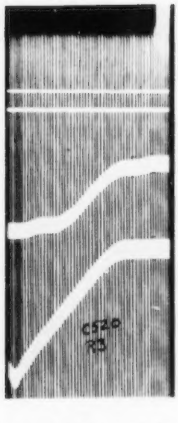


Fig. 5. Reproduction of record 3 C 520 read from right to left. The time lines which run vertically are $\frac{1}{10}$ second apart. The blackened portion at the top indicates the duration of the stimulus. The upper horizontal line is the base line from which measurements of displacement were made. The second horizontal line is the torsion wire myograph which was not used in this record. The third line shows the shortening of the muscle. The fourth line records the displacement of the disc. The "give" in the apparatus is more evident in the muscle than in the disc record.

the lightest equivalent mass, 12.8 kgm. and they show the highest initial tensions which were developed in the series of experiments. These tensions were, for the four experiments cited above, 17.8 kgm., 20.1 kgm., 15.01 kgm., 17.9 kgm. respectively. In form, these curves are concave upward, due to the small initial decrement. No sufficient explanation has been found for this discrepancy.

The slope of each of the twenty-nine curves has been determined by drawing a straight line through the points in such a way as to come closest to the greatest number of them. The angle formed by this line and the x-axis has been expressed by the ratio of the force decrement to the speed of shortening. In table 3 these slopes are distributed with respect to the equivalent masses from which they were obtained. It is evident that there is a most frequent slope for each equivalent mass. This slope averages 25° with the 12.8 kgm. equivalent mass, 35° with the 137.6 kgm. and 55° with the 337.4 kgm. equivalent mass. This fact is shown graphically in figure 4.

It may be concluded from these results, within the limit of error of the experiments and over a certain portion of any given contraction that 1, the force decrement varies in a linear manner with increasing speed of shortening (with the four exceptions noted above); 2, the slope of this curve increases with an increase in the equivalent mass, and 3, this curve passes through the origin of the axes.

Discussion. The fact that a contracting muscle loses force when it shortens was known to A. Fick who attempted to explain the facts of muscle dynamics on the theory that contracting muscle behaves like an elastic body. Fick demonstrated by means of his Schwunghebel (inertia lever) that a part of the force of an elastic body such as a steel spring, rubber band and, to a greater extent, muscle, is used up in overcoming its own internal resistance to change of form.

The same conception and essentially the same apparatus were, later, independently applied by A. V. Hill (3) to a study of the problem. Hill proposed that the decrease in force might be predicted "with certain simple assumptions as to the viscous resistance of the muscle to change of form." These assumptions are that

> The change in form of the muscle involves the flow of fluid through the protoplasmic or colloidal network and the visible structures of the cells, and a given change in form is similar, in general character, to the flow of a given amount of fluid through a set of capillary tubes. Thus the mechanical energy degraded into heat should be proportioned to the rate at which the given change in form is caused. Suppose a muscle, when undertaking a maximum contraction, to possess mechanical potential energy Wo; then if the shortening be against the reaction of an almost infinitely great mass, it will be very slow, and practically the whole of the mechanical energy will be realized as work; if, however, the mass providing the reaction be finite, the change of form will proceed at a finite rate, and a finite proportion of the mechanical energy will be degraded into heat; only the remainder, W, say, being realized as work. Thus

(Wo–W) is equal to the energy dissipated in the shortening, which from above, we should expect to be proportional to the velocity with which the shortening is carried out (p. 28).

Our results conform to this expectation in part. There are, however, two points in which the facts diverge from the theory. Figure 3 shows that the ratio of force decrement to speed of shortening, which may be called "apparent viscosity" is fairly constant until the contraction of the muscle approaches completion, when it shows a decided decrease—the force exerted on the inertia becomes greater than that predicted. The same fact is shown in the shape of the tension-length diagram of figure 1. The lower ends of the isotonic curves tend to parallel the x-axis. In view of the fact that the apparent viscosity does not decrease until the end of the contraction, it may be assumed that a considerable number of fibers have undergone relaxation by that time and become excitable again. Since the stimulus acts continuously throughout the contraction, it may be suggested that some of the relaxed fibers are reëxcited, resulting in the redevelopment of a certain measure of force. The relative increase in force at this point over that predicted by the theory can be accounted for in this manner. A second divergence of our results from the theory occurs in the increase of the slope of the force decrement speed curve with increased equivalent mass, which is shown in figure 4. With viscosity as the sole factor effective in reducing the force of the muscle, when it shortens, there should be no change in the slope (viscosity constant) with increase in equivalent mass. This fact expressed in arbitrary units was found by Gasser and Hill (4, fig. 2, p. 403), with the double sartorious preparation of the frog. Whether it is possible to interpret our results from the standpoint of the damped and free elasticity concepts employed by Levin and Wyman (5) has not yet been determined.

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THE RESISTANCE OF SURVIVING SPINAL ANIMALS TO HYPOGLYCEMIA INDUCED BY INSULIN

CHANDLER M. BROOKS[1]

From the Laboratories of Physiology in the Harvard Medical School

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The observations of Sherrington (1906), and others (Tigerstedt, 1923) upon the gradual recovery of vascular tone and pressor reflexes after cervical transection of the cord reveal the presence of a spinal vasoconstrictor mechanism. These vasomotor reactions involve the sympathetic system and show that sympathetic activity can be elicited from a spinal animal. Recently (Brooks, 1933) a study was made of the reflex sympathico-adrenal activity which could be aroused in the spinal cat by nociceptive stimuli. It was found that weak afferent stimulation of the cut sciatic nerve produces a rise in blood sugar, increments in arterial pressure and heart rate, diminished clotting time and contraction of the denervated nictitating membrane. These changes, though not as great as those which the same stimuli would have produced had the medulla and higher centers not been removed, were definite enough to indicate moderate sympathico-adrenal excitation. The question next arose as to whether or not other methods of exciting sympathico-adrenal activity in the normal cat would produce a similar activity in the spinal cat.

Cannon, McIver and Bliss (1924) found that insulin hypoglycemia produces strong sympathetic activity in normal animals. This work proved that the sympathico-adrenal system is part of the mechanism protecting the body from dangerous hypoglycemia and that denervation of the adrenals renders animals less resistant to insulin. This has been frequently confirmed by other workers. Britton, Geiling and Calvery (1928) denervated the adrenals and found that sensitivity to insulin increased four- to ten-fold. Such animals had lost their ability to recover spontaneously from the hypoglycemia induced by very small amounts of insulin. Dworkin (1931a) and Schlossberg, Sawyer and Bixby (1933) found the same to be true for completely sympathectomized animals. The general conclusion drawn from these experiments is that during insulin hypoglycemia there is an augmented medulliadrenal secretion, stimulated centrally or reflexly and mediated by the sympathetic system.

[1] Medical Fellow of the National Research Council.

Since insulin hypoglycemia excites sympathico-adrenal activity in normal animals, it became of interest to ascertain whether or not such activity can be similarly induced in the spinal cat. If the activity of the thoracolumbar sympathetic system is entirely controlled by centers located in the brain, transection of the cord in the cervical region should render animals devoid of any compensatory protective sympathico-adrenal discharge and as sensitive to insulin as are completely sympathectomized and adrenal-inactivated preparations.

METHOD. Transections of the spinal cord at the level of the sixth cervical vertebra were performed aseptically under pentobarbital sodium (0.7 cc. per kgm.) anesthesia. These cats were kept about a week before being used in the experiments. That was a sufficient time for the recovery of vascular tone and the return of arterial pressure to normal. Vigorous crossed-extension and flexion reflexes could easily be obtained from these animals. Their temperature was maintained at a normal level and they were catheterized at 24-hour intervals. Every day each cat received, by stomach tube, 20 grams of dextrose dissolved in 100 cc. of milk, and occasionally they could be persuaded to eat small pieces of meat. No food was given during the twelve hours previous to the experiments.

The adrenals were inactivated by removal of the right and denervation of the left. Denervation of the heart was performed as described by Cannon, Lewis and Britton (1926). The left vagus was always cut at the beginning of the experiment to preclude the possibility that branches to the heart might have been missed during the denervation and still exert some influence. All animals were allowed to recover from the abdominal and thoracic operations before their cords were transected.

Changes in arterial pressure and heart rate were ascertained by means of a cannula attached to the left femoral artery and recorded by a mercury manometer. Samples for blood-sugar assay were obtained from a cannula inserted in the right femoral artery just distal to the saphenous branch. The Folin-Svedberg (1930) method of sugar determination was used and duplicate samples were always secured. No blood-pressure records were taken in cats with denervated hearts, the heart rates being determined by means of a stethoscope and the blood samples being obtained from an ear vein. Blood samples were taken, and heart rate and arterial pressure were determined at 20-minute intervals. Determinations were begun an hour before the subcutaneous injections of insulin. No anesthesia was necessary except for manipulations such as cutting the vagus in the neck. Local anesthesia was then used.

RESULTS. The results of this series of experiments have been summarized in table 1. The sensitivity of normal cats to insulin is so variable, even in the same animal at different times, that it is almost impossible to say what dosage of insulin will produce slight hypoglycemic symptoms,

convulsions or death. McCormick, Macleod, Noble and O'Brien (1923) have shown that the nutritional condition of an animal is of cardinal importance. The general state of health also has a profound effect upon

TABLE 1

CAT	INSULIN	BLOOD SUGAR		TIME TAKEN TO REACH LOWEST LEVEL	TERMINATION OF THE EXPERIMENT	BLOOD SUGAR AT TERMINATION OF EXPERIMENT	TIME FOR RECOVERY OR DEATH AFTER INSULIN
		Before giving insulin	Lowest level after insulin				
	units per kgm.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*minutes*			*minutes*
Cord cut but otherwise normal							
42	4	65	30	96	Death	30	96
44	2	80	40	100	Death	45	120
45*	1.5	60	24	150	Death	34	160
47	1.5	100	19	150	Death	28	210
40*	1.5	120	32	140	Recovery	130	300
41*	1	72	36	180	Recovery	110	360
42	1	68	22	240	Death	33	300
50*	1	66	26	180	Recovery	80	420
50*	1	70	18	160	Recovery	78	360
40	0.5	70	30	150	Recovery	76	270
48*	0.5	70	24	210	Recovery	84	360
49*	0.5	80	30	180	Recovery	82	300
49*	0.5	80	20	180	Recovery	78	300
Cord cut—decerebrated (pituitary removed)							
51	0.5	100	38	120	Recovery	74	300
54	0.5	78	32	120	Recovery	68	270
Cord cut—chronic heart denervation							
5*	0.5	58	18	150	Recovery	64	420
9*	0.5	75	18	130	Recovery	52	300
9*	0.5	61	24	150	Recovery	50	360
10*	0.5	88	17	300	Recovery	68	390
10*	0.5	68	14	150	Recovery	66	420
Cord cut—adrenals inactivated							
52	0.5	74	28	60	Death	28	60
56	0.5	68	26	60	Death	26	60
60*	0.5	90	18	80	Death	18	80
61	0.5	80	28	90	Death	28	90
71*	0.5	58	16	110	Death	16	110
Normal animal—adrenals inactivated							
8	0.5	58	12	180	Death	12	180
11	0.5	64	19	140	Death	19	140

* Vagi cut.

an animal's resistance to insulin. From the large number of tests reported (Cannon, McIver and Bliss, 1924; Britton, Geiling and Calvery, 1928; Dworkin, 1931b; Schlossberg, Sawyer and Bixby, 1933; etc.), it can be seen

that frequently an animal shows only mild symptoms when given as much as 4 units of insulin per kgm. Often, however, cats go into convulsions when only 1 unit per kgm. is injected. But it can be said that in general 1 unit per kgm. induces definite hypoglycemic symptoms and that 2 to 3 units per kgm. produce convulsions. In the present experiments cat 40, while normal, developed practically no symptoms when given 2 units per kgm.; after its cord was cut it recovered from 1.5 units per kgm. On the other hand, cats 42 and 5 went into convulsions when only 1 unit per kgm. was injected, though they appeared to be in excellent physical condition. After cord transection 1 unit per kgm. was fatal to cat 42, and the effect of 0.5 unit per kgm. on cat 5 was very profound and recovery was unusually slow. It can be seen from table 1 that as the dosage of insulin was reduced the animals either lived for a longer time than those given larger doses or recovered completely within five or six hours. Two cats which were not able to recover fully from 1.5 units of insulin per kgm. exhibited some compensatory ability, for the blood sugar had begun to rise before death. Evidently the carbohydrate reserve was not sufficient or the forces tending to raise the glycemic level were not sufficiently powerful to restore completely the blood sugar to normal. The majority of cats recovered from 1 unit per kgm., and they all survived 0.5 unit. Four animals withstood a second injection of insulin. Though it is apparent that the resistance of these spinal cats to insulin is seriously impaired, their resistance is much greater than animals with adrenals inactivated.

The papers previously referred to furnish abundant proof that sympathectomized or adrenal-inactivated animals can never recover spontaneously from 0.5 unit of insulin per kgm., and 0.3 or 0.1 unit per kgm. is frequently fatal. Spinal cats with inactivated adrenals are no exception to the rule, for an injection of 0.5 unit per kgm. invariably resulted in death within an hour or two (table 1). The two animals without cord transections which had had their adrenals denervated were slightly more resistant to insulin, probably because of their better nutritional condition, and they withstood the hypoglycemia for a slightly longer time. Though the adrenal medulla seems to be the sole source of resistance to insulin in these spinal cats, to make sure that the pituitary was not involved (cf. Olmsted and Logan, 1923) two animals were decerebrated and the pituitary removed. Both recovered spontaneously from the insulin injections.

It was thought that the denervated heart might furnish direct proof of the liberation of adrenin due to critical hypoglycemia in these spinal animals. Three cats were prepared for survival after denervation of the heart. Their normal reaction to 0.5 unit of insulin per kgm. was ascertained; their cords were transected and after a few days the same amount of insulin was again injected. Unfortunately the insulin or the hypoglycemia produced a definite slowing of the heart rate and although, in all cases, the

heart ultimately accelerated and the rate rose above the initial level, this direct insulin effect somewhat impaired the value of the denervated heart as an indicator of adrenal secretion. Dworkin (1931b), on the basis of his own work and a summary of the literature, reported that insulin does reduce the heart rate provided the effect of the sympathetic system is excluded. In normal animals, after heart denervation, sympathetic discharge was great enough to overcome the insulin effect, but in the spinal state apparently it was some time before the adrenin reached a sufficient concentration to overcome the depressant effect of the insulin, and the acceleration was somewhat delayed and possibly reduced.

Spinal animals with vagi and accelerators intact frequently showed marked slowing of the heart during the most severe periods of hypoglycemia. This invariably resulted in an abnormally low arterial pressure. Occasionally a drop in arterial pressure occurred which was not accompanied by diminution of the heart rate. Possibly a change in the strength of the beat or a loss of peripheral vascular tone due to some hypoglycemic effect on the cord or blood vessels was responsible. Recovery of a normal glycemic level in the animals was always accompanied by a return to normal of the heart rate and arterial pressure. In the majority of cases (table 1) the blood sugar rose even above the initial level and occasionally this was true of heart rate also. The heart rate and blood pressure of the adrenal-inactivated cats dropped along with the blood sugar, and in these animals the fall was not checked as in the other spinal preparations. This resulted in a very low pressure which, along with the deficient heart action, may have been largely responsible for the respiratory failure and death.

Olmsted and Logan (1923) reported that insulin convulsions never occur in decapitate cats. This was confirmed by Kleitman and Magnus (1924), but Olmsted and Taylor (1926) later claimed that typical insulin convulsions can be elicited from spinal preparations. Best, Hoet and Marks (1926) mention the occurrence of muscular spasms in insulinized decapitate cats similar to those observed by Olmsted and Taylor, but they state that these spinal preparations do not show typical convulsions and that the muscular activity noted does not deplete the muscle glycogen as do the hypoglycemic convulsions of normal animals. None of the animals observed during this series of experiments ever showed typical hypoglycemic convulsions. They generally passed through a stage of hyperexcitability, however, and slight muscular twitching was frequently seen.

Using the denervated heart as an indicator, Cannon, McIver and Bliss (1924) showed that there apparently are two levels of resistance to hypoglycemia induced by insulin. When the blood sugar reaches a critically low level the sympathetic system becomes active, the adrenal medulla discharges adrenin into the circulation, and the denervated heart accelerates. If this adrenal activity is not sufficient to check the drop in glycemic

percentage convulsions ultimately result and are accompanied by a more profound sympathetic activity as evidenced by further acceleration of the heart. If the occurrence of convulsions in the normal cat is in any way responsible for the additional sympathetic activity this explains in part the inability of spinal animals, in which convulsions do not occur, to withstand doses of insulin which tax the resistance of normal animals. It has been my observation that any dose of insulin which produces convulsions in a normal cat will kill that animal after cord transection.

Although convulsions are eliminated by cervical transection of the cord, other hypoglycemic symptoms are exhibited. Mewing, salivation, pupillary dilatation and defecation occur. Convulsive movements of the head appear, and the respiration is greatly accelerated. The paralysis of the intercostal muscles makes true panting impossible, but frequently the respiratory rate rises from an initial frequency of 20 to 48 or 50 a minute, and the animals exhibit a diaphragmatic panting, breathing with mouth open.

The general conclusions which may be drawn from these results are several in number. Spinal animals are not able to recover spontaneously from quantities of insulin which tax the resistance of normal animals. These animals do possess some ability to compensate for a low blood sugar. This resistance must be due to definite sympathico-adrenal activity elicited by the hypoglycemia, for cutting the sympathetic supply to the adrenals completely abolishes the ability of cats to recover from doses of insulin which spinal animals with innervated adrenals can easily endure. Other evidence that the sympathetic system and the adrenal medulla are involved in checking hypoglycemia induced by insulin is furnished by the fact that the normal and denervated heart rates as well as the blood sugar eventually rise well above the initial level, while in animals without innervated adrenals the depressant effect of the insulin or hypoglycemia is not counteracted and retardation is not checked.

SUMMARY

Surviving spinal animals with the cord transected in the cervical region have a diminished resistance to insulin.

Hypoglycemia activates the sympathetic system of a spinal cat to such an extent that 0.5 unit of insulin per kgm. is never fatal, and usually a cat can recover from 1 unit per kgm.

Spinal animals in which the adrenals have been inactivated cannot recover spontaneously from 0.5 unit of insulin per kgm.

Convulsions are eliminated by cervical transection of the cord though other hypoglycemic symptoms occur.

These observations indicate that a hypoglycemia induced by insulin which does produce strong sympathico-adrenal activity in the normal cat

can likewise induce activity of this system in a spinal cat in which all nervous connections between higher centers and the spinal thoraco-lumbar sympathetic outflow have been cut.

The results afford further evidence for the existence of a spinal mechanism capable of exerting some influence upon the peripheral sympathico-adrenal system.

I wish to express my indebtedness to Prof. W. B. Cannon and Prof. Philip Bard for the assistance which they have so generously given.

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THE RELATION OF BILE TO THE SECRETION OF PANCREATIC JUICE

LESTER R. DRAGSTEDT AND ROBERT A. WOODBURY

From the Department of Surgery of The University of Chicago

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About forty years ago Dolinski (1894) observed that the application of dilute acids to the mucous membrane of the duodenum caused a profuse secretion of pancreatic juice. These findings were confirmed by Popielski (1901) who believed that the pancreatic response was due to a local reflex, involving the duodenum and the pancreas. In 1902, Bayliss and Starling formulated the theory that the secretion of pancreatic juice was brought about by a humoral or hormone mechanism. This theory was based upon experiments which showed that the introduction of a dilute hydrochloric acid solution into an isolated denervated segment of jejunum or the intravenous injection of an acid extract of the duodenal mucosa would cause pancreatic secretion. The specific excitant to the pancreatic cells was termed secretin. It was assumed to exist in the duodenal mucosa in an inactive precursor form, prosecretin, which was changed to the active secretin and discharged into the circulating blood when the hydrochloric acid of the gastric content came into contact with the duodenal mucosa. While the major tenets of the secretin theory of Bayliss and Starling have been confirmed (Weaver, Luckhardt, and Koch, 1926; Ivy, Farrell and Lueth, 1927) some question has been raised concerning the existence of prosecretin. This literature has been reviewed by Still (1931).

In a series of recent papers, Mellanby (1925, 1926, 1928) has undertaken a critical examination of certain of the postulates of the secretin theory in the light of his own experiments. He concluded that secretin "exists in a preformed condition in the mucous membrane of the small intestine since active solutions of it may be obtained by solvents so diverse as water, 5 per cent NaCl, 0.85 per cent NaCl, 2 per cent HCl, 0.1 per cent NaOH, 75 per cent alcohol and 75 per cent acetone." That being the case and assuming that secretin was immediately responsible for the secretion of pancreatic juice he maintained that some definite event must cause the passage of this secretin from the cells of the duodenal mucosa into the portal blood. The fact that secretin could be obtained from intestinal mucous membrane which normally never comes into contact with acid, that pancreatic secretion apparently occurs in a normal manner in persons with achlorhydria

and after gastric resection, and that the pancreatic response to the application of acid to the duodenal mucosa does not equal the response to the intravenous injection of secretin preparations all have led him to seriously question the importance of acid as a pancreatic stimulus. "It is evident, therefore, that there exists no immediate causal relation between gastric acidity and pancreatic secretion." In a series of experiments, performed on cats anesthetized by urethane, Mellanby found that the introduction of bile of the proper reaction into the duodenum caused a copious secretion of pancreatic juice. The activity of the bile was found to be due to cholic acid and its compounds. These facts were harmonized with the theory of

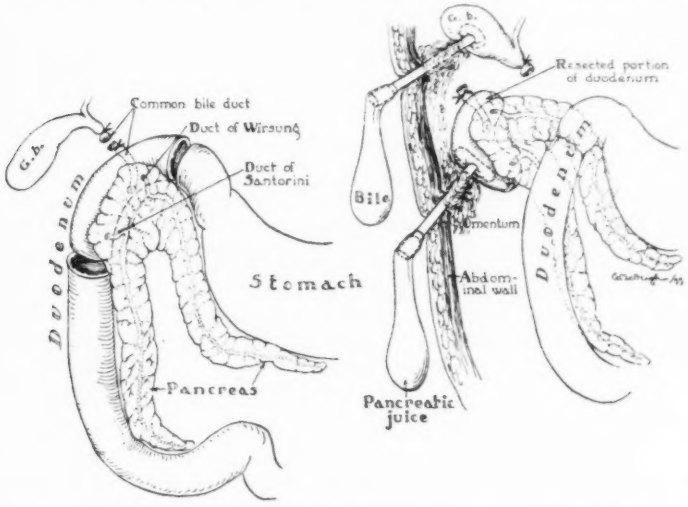

Fig. 1. Diagram illustrating method for producing total biliary and pancreatic fistulae. In each case the gold plated cannulae were carefully wrapped with omentum before being led through a stab wound in the abdominal wall.

Bayliss and Starling in the following way. "The immediate stimulus for the secretion of pancreatic juice is secretin. Secretin contained in the cells of the intestinal mucosa is carried into the portal blood associated with the bile salts contained in the fluid absorbed from the intestine. The facts offer an adequate basis for the appreciation of the severe digestive disturbances which occur in catarrhal jaundice." In addition this theory of Mellanby would offer an adequate explanation for the development of chronic penetrating ulcers in the duodenum of experimental animals provided with biliary fistulae. It is well known that the removal of pancreatic juice from the duodenum is shortly followed by the development of progressive ulceration (Matthews and Dragstedt, 1932) which may be ac-

counted for by the corrosive action of the free hydrochloric acid of the unneutralized gastric content. Bile however can effect but little neutralization of the gastric juice and it is difficult to see why its removal from the duodenum should lead to ulceration unless as suggested by Mellanby there occurs an associated diminution or cessation of pancreatic secretion.

The recent development in our laboratory of methods for the production of permanent biliary and pancreatic fistulae (Dragstedt, Montgomery, and Ellis, 1930) has made it possible to subject this problem to what seems

TABLE 1

Effect of removal of bile from the intestine on the secretion of pancreatic juice in a dog with a pancreatic and biliary fistula

	DATE, 1933	PERIOD OF COLLECTION	RINGER'S SOLUTION, INTRAVENOUS	MATERIAL ADMINISTERED BY MOUTH					BILE	PANCREATIC JUICE	ALKALINITY OF PANCREATIC JUICE
				Water	Milk	Bread and meat	Pancreatic juice	Bile			
		hours	*cc.*	*cc.*	*cc.*	*grams*	*cc.*	*cc.*	*cc.*	*cc.*	
Dog 1	3–19	72	5000	1400	2300	162	1610	440	450	1962	106
	3–22	72	6000	2550	2390	63	1190	290	242	2647	111
	3–25	72	5500	2634	2135	920	2000	0	227	3344	124
	3–28	72	3000	2450	650	1054	2400	0	189	2654	136
	4– 3	72	4000	1980	800	492	2400	300	472	2579	140
	4– 6	72	4500	1140	1920	294	2400	300	280	1392	152
	4– 9	72	3700	1760	1100	318	2400	0	213	2703	139
	4–12	72	3000	2400	850	226	2210	0	213	1985	154
Dog 2	6–24	72	7000	2658	3200	304	2903	617	669	2982	
	6–27	72	6500	2470	3400	496	3028	469	529	3143	
	6–30	72	3500	3000	4330	772	3139	0	468	3324	
	7– 3	72	3000	3280	3290	1312	3556	0	Lost	3422	
	7– 6	72	3000	2910	2770	1540	3934	704	839	4340	
	7– 9	72	3000	3400	2950	1585	4170	1151	1161	3774	
	7–13	72	3000	5650	2710	1552	3860	0	548	4132	
	7–16	72	2000	4600	3000	1563	4172	0	505	4004	
	7–20	72	0	7630	2900	1524	4056	388	378	3669	
	7–23	72	0	7780	2850	1500	2797	361	351	2502	

to us a crucial test. Two dogs under complete ether anesthesia were operated upon as illustrated in figure 1. The duodenum was cut across about 3 cm. distal to the pylorus and again about 1 cm. below the lower pancreatic duct. The continuity of the intestine was re-established by end to end anastomosis between the proximal and distal duodenum. The common bile duct was divided between ligatures. The ends of the isolated segment of duodenum were infolded and closed. A gold plated cannula, previously described (Dragstedt, Haymond, and Ellis, 1933) was placed

in this pancreatic-duodenal sac and also in the fundus of the gall bladder. The animals recovered uneventfully from the operation and after a few days the experiments summarized in table 1 were carried out. It should be emphasized that by this method a quantitative collection of all bile and pancreatic juice secreted in 24 hours could be secured by means of sterile rubber balloons attached to the cannulae. A record was kept of the amount of bile and pancreatic juice secreted each 24 hours, as well as the amount and kind of food ingested. As a rule all of the bile and pancreatic juice secreted during the previous day were returned by means of a stomach tube passed at two hour intervals between 8:00 a.m. and 6:00 p.m. To shorten the table the data are indicated for consecutive periods of 72 hours each showing the volume of pancreatic juice secreted during 6 days when all the bile secreted was returned to the alimentary tract and then for 6 days when no bile was present in the intestine. In the first test animal 1 secreted 4609 cc. of pancreatic juice and 692 cc. of bile in 6 days when practically all of the bile and pancreatic juice were returned to the alimentary tract. During the succeeding 6 days no bile was returned and 5998 cc. of pancreatic juice and 416 cc. of bile were secreted. In the second test 3971 cc. of pancreatic juice and 752 cc. of bile were secreted in 6 days when both secretions were returned to the stomach whereas in the succeeding 6 days when only the pancreatic juice was returned, 4688 cc. of pancreatic juice and 426 cc. of bile were secreted. The results obtained with animal 2 were essentially similar. In each case the dog secreted a little more pancreatic juice when no bile was present in the intestine than when it was returned by mouth. Considerably less bile however was secreted, a finding in harmony with the theories of Schiff (1868) regarding the circulation of bile.

These findings seriously question the interpretation Mellanby placed on his own experiments. In the dog, at least, it is quite evident that the absence of bile from the duodenum does not prevent or diminish the secretion of pancreatic juice in response to the ingestion of food. Bile salts cannot therefore be considered essential either for the activation of the specific pancreatic stimulant or for its passage into the blood in effective form.

SUMMARY

Total biliary and pancreatic fistulae were prepared in each of two dogs. The volume of pancreatic juice and of bile secreted during 6 days under standard diet when both secretions were returned to the stomach was determined and compared with the volumes secreted when only the pancreatic juice was returned. The complete removal of bile from the intestinal tract caused a distinct decrease in the volume of bile secreted but had no effect on the volume of pancreatic juice. It was concluded that in the

dog at least the presence of bile in the duodenum cannot be considered essential for the secretion of pancreatic juice.

These observations are in harmony with those of Ivy, who found that ligature of the common bile duct in the dog did not decrease the secretion of pancreatic juice in response to the ingestion of meat. (Ivy, A. C. The Physiology of External Pancreatic Secretion. Journ. A. M. A. 1927, Vol. 89, p. 1030.)

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STUDIES ON THE PHYSIOLOGY OF SLEEP

XI. Further Observations on the Effects of Prolonged Sleeplessness

N. R. COOPERMAN, F. J. MULLIN AND N. KLEITMAN

From the Department of Physiology of the University of Chicago

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Deprivation of sleep usually results in a feeling of decreased efficiency in the performance of the daily tasks. This study was undertaken in order to investigate the condition resulting from the abstinence from sleep by certain methods not heretofore used, as well as the characteristics of the sleep following such a period of prolonged wakefulness by the methods recently described by us (Kleitman, Cooperman and Mullin, 1933).

In our present work the procedure followed was somewhat similar to that employed by Lee and Kleitman (1923). The tests were made on six male subjects each of which underwent one or more sleepless periods of approximately 60 hours' duration, a total of 13 such periods. The subjects were in good health at the time of the experiments and suffered no ill effects from their experience.

Procedure. During the period of sleeplessness conditions were kept uniform, and muscular activity was maintained at a minimum compatible with wakefulness. The various tests were performed regularly at 7 a.m., 4 p.m. and 9 p.m., and irregularly at other hours. Control tests were made at these same times for several days preceding and following the insomnia periods. For the entire duration of wakefulness three or four observers would take turns in watching the subjects so that the watchers were always wide awake.

The tests that had been used previously and some which we modified were:

1. The ability to stand upright with eyes closed. The inevitable swaying of the body, in the course of two minutes, was recorded by a device similar to the one used by Lee and Kleitman (1923).

2. The time taken to name 100 colored squares, pasted on a cardboard, in ten rows of ten. This was run through four times, the score being kept for each successive 100 colors as well as for the whole 400. The number of uncorrected errors and corrected errors was also noted.

3. Reaction time to sound stimuli (simple) and to light (red-green choice) was measured by a Hipp chronoscope.

The above tests were employed mainly to note whether or not the data obtained were in agreement with the results of the following new tests:

1. Hand steadiness, as determined by the ability to hold a metal stylus in one of several holes of varying diameter in a metal plate, without touching the edges of the hole (Whipple, 1914). Any contact between the stylus and the plate closed an electric circuit which actuated a counter. Scoring was based on the number of contacts made in three one-minute trials separated from each other by one minute of rest.

2. Visual acuity, as tested by showing the subject cards containing single and double lines and asking him to differentiate between them from a given distance, depending on the eyesight of the individual.

3. The threshold of cutaneous sensibility to touch and pain, as measured by means of the sets of hairs of von Frey (1926). In this test five to eleven spots distributed over the skin of the face, ear, wrist and fingers were picked at random and marked with India ink, so that the same spots could be stimulated repeatedly.

RESULTS. Subjectively it was easily ascertained that it was much more difficult to keep awake, as well as to perform the various tasks of the tests, between 3 and 6 a.m. than later in the following day. A more detailed study of the records showed that the subject's performance was influenced not only by the time of the day or night, but also by what he had been doing during the half-hour or so preceding the test.

Some of the subjects became hyper-irritable and reacted in an uncalled-for manner at the slightest provocation. During the latter part of this period the subjects would tend to act as if they were dreaming and would often stare into space, particularly during the performance of the hand-steadiness test. Frequently their behavior resembled that of persons who were under the influence of an intoxicating dose of alcohol.

On going to bed after a period of sleeplessness, the subjects fell asleep almost imme 'iately. On the following day a majority of them did not feel as wide-awake as usual.

Objectively, as was reported by previous observers, we found no significant changes in reaction time or the ability to name 100 colors, but marked changes in the ability to stand upright with one's eyes closed. In recording the time required to name successive 100 colored squares in a 400-color test, we observed a slowing up in the naming of the third and fourth hundreds only.

As regards the new tests, visual acuity did not show any consistent deviation from the normal, but there was a decrease in hand steadiness on the third day of the period of wakefulness. Striking results were obtained, however, with the von Frey hairs, as can be seen from a typical series of figures, given in table 1. While the threshold for touch sensitivity remained practically unchanged (and therefore not shown in the table), the

TABLE 1

The effect of prolonged wakefulness upon cutaneous sensitivity to pain

REGIONS STIMULATED	INTENSITIES OF THRESHOLD STIMULI										
	Control tests before prolonged wakefulness			Successive tests at the following hours after the beginning of the period of wakefulness							Control tests after prolonged wakefulness	
				24	26	29	35	39	47	50		
Forehead	10	10	10+	10+	10+	8	8	6	6–8	6–8	10+	10+
Nose	8	8	4	8	6	4	4	4	4	4	8	10+
Angle of mouth	10+	8	10	10+	8–10	4	4–6	2–4	2	2	8	8
Cheek	10	10+	6	8	8	6	2	4	2–4	4	10+	10+
Chin	10	10+	6	10+	6	4–6	6	6	4	4	10+	10+
Ear	10+	8–10	10	10+	8	6	6	8	6	4	10+	10+
Neck	10+	10+	10+	10+	10+	6	6	6	4–6	4	10+	10+
Finger	10+	10+	10+	10+	8	6	6	6	4	6	10+	10+
Wrist	10+	10+	10	8	8	8	8	6–8	6	8	10+	10+
Corner of eye	10	10	10	8	8	6–8	6–8	6–8	4	4	10+	10+
Upper lip	1	2	1	$\frac{3}{4}$	1	1	$\frac{3}{4}$–1	1	1	1	1–2	2

The above values were obtained before, during and after one period of prolonged wakefulness on subject F. J. M. and are typical of the results obtained on other subjects. The figures standing for the intensities of the threshold stimuli, the lower the figures, the greater the irritability.

TABLE 2

The effect of prolonged wakefulness on motility during the supervening sleep

SUBJECTS	NUMBER OF PERIODS OF WAKEFULNESS	HOURS OF SUPERVENING SLEEP	MOVEMENTS PER HOUR		TIME SPENT IN MOVEMENT IN SECONDS PER HOUR		CM. OF DISPLACEMENT OF BEDSPRING PER HOUR	
			Control	Experimental	Control	Experimental	Control	Experimental
A. I. D.	3	11.0 12.9 10.1	2.9	1.5	29.5	19.2	12.2	2.4
N. B.	3	12.5 12.1 12.2	4.4	2.8	39.5	21.4	11.8	6.3
F. J. M.	2	10.3 10.0	5.1	1.8	20.3	21.9		
N. R. C.	2	13.5 13.6	5.8	3.5	39.1	28.3	12.1	6.5
E. L. B.	2	10.8 11.3	6.9	3.4	48.3	22.4	10.4	4.3
J. W.	1	12.5	7.8	1.8	34.0	20.0		

threshold for pain sensitivity was greatly reduced in every one of the six subjects, on every abstention from sleep.

The sleep following a period of wakefulness of 60 hours was characterized by a considerable increase in duration, a marked decrease in motility, both as regards frequency, extent, and duration of movement (table 2). A comparison between the record of motility during a typical night's rest and that obtained during the sleep that followed a period of prolonged wakefulness is shown in figure 1. The body temperature level on control nights was, on the average, 0.4°F. higher for the first two hours, and for the remainder of the night 0.6°F. lower, than on nights following a period of prolonged wakefulness. Individually the temperature curves during sleep following sleeplessness were characterized by a lack of uniformity.

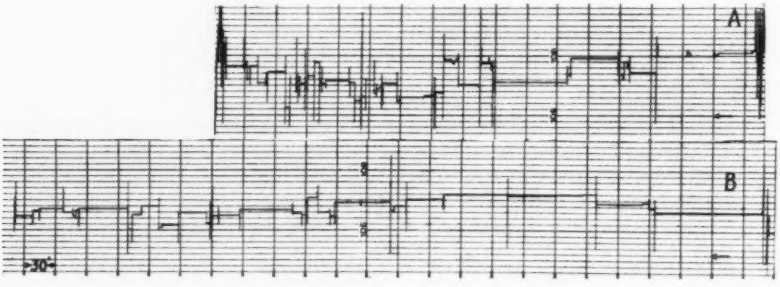

Fig. 1. Typical records of motility during sleep obtained on subject N. B. *A*, control night's sleep of over eight hours' duration, and *B*, a longer night's sleep following a period of wakefulness of 62 hours. There were fewer movements per hour and the phase of increased motility began much later in *B* than in *A*.

Discussion. The various tests used, if they involved mental or muscular activity of very short duration, did not reveal any variation from the normal as a result of abstaining from sleep. As was shown by Lee and Kleitman (1923) for color naming, increasing the time required to carry out a task brought about a much poorer performance than normal. In this series, timing the naming of successive four one hundred squares, we found that the slowing up commenced with the third hundred, and was worse during the fourth. As the period of sleeplessness progressed the number of corrected errors became fewer and the number of uncorrected errors increased, which indicates that there was a tendency on the part of the subjects either not to notice or to disregard mistakes already made.

Steadiness was found to be more closely related to the time of the day than to the number of hours after the beginning of the period of wakefulness. The diurnal variation in steadiness reported by Kleitman (1933) may have overshadowed the possible effect of loss of sleep.

During the latter part of the period of wakefulness the subjects' general behavior was in some respect similar to that caused by the consumption of a large dose of alcohol, such as 1 cc. per kilogram of body weight. This was brought out particularly in the body steadiness test. In respect to changes in sensitivity to pain, the effect was just the opposite: loss of sleep increases sensitivity to painful stimulation, whereas alcohol, according to as yet unpublished observations of Luckhardt and Mullin, dulls it. It is interesting to note that sensitivity of the skin to touch stimulation is not affected in either condition.

The duration of sleep, when the subjects are permitted to sleep ad libitum after a period of prolonged wakefulness, is definitely greater than on control nights. It amounted, on the average, to twelve hours, and in four out of five cases it was of very nearly the same length after each period of wakefulness (table 2). The decreased motility may be responsible for the fact that a majority of the subjects did not feel as rested upon arising as they usually did. One subject remained almost perfectly quiet for over four hours, something that never happened on an ordinary night. The decrease in motility is perhaps due to a decreased sensitivity to discomfort which would ordinarily lead to body movements. That the increase in motility which usually manifests itself after about four hours of sleep on a control night does not start until about six hours of sleep, following prolonged wakefulness (fig. 1), points in that direction.

SUMMARY

1. During a period of prolonged wakefulness (60 hours) there is developed a marked increase in cutaneous sensitivity to painful stimulation, while there is no change in sensitivity to touch.

2. Individuals sleep from eleven to thirteen hours after sixty hours of sleeplessness, instead of the usual eight to nine hours.

3. The sleep following such a period of wakefulness is characterized by a decrease in frequency of movement, time spent in stirring, and the sum total of movements.

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EXPERIMENTS ON THE CHEMICAL STIMULATION OF PAIN-ENDINGS ASSOCIATED WITH SMALL BLOOD-VESSELS

ROBERT M. MOORE, RUTH E. MOORE AND ALBERT O. SINGLETON, JR.

From the Department of Surgery, University of Texas, Medical Branch, Galveston

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In animals under sodium-amytal anesthesia the stimulation of afferent neurons subserving pain-sensibility results in reflex motor activity of a pseudaffective nature. In an earlier communication (Moore and Moore, 1933) attention was called to the occurrence of such reflex activity following the intra-arterial injection of various solutions. Experiments which were reported indicated that the smaller vascular branches are supplied with numerous pain-fibers and that the endings of these fibers are stimulated when irritating solutions are injected by way of the parent-vessel. The investigation has been continued to determine the chemical properties which may render a solution irritating to these pain-endings.

METHOD. The experiments were performed upon cats under sodium-amytal or dial anesthesia. Injections were made into the femoral and brachial arteries. The vessel was ligated and the solution, at room-temperature, was injected peripheral to the ligature. The occurrence of any visible response to the injection was noted and its character and time relations were described.

At first the volume of the injection was arbitrarily set at 0.5 cc. However, in such quantity, a solution of greater concentration was required to elicit reactions from the femoral artery than from the brachial. This proved to be due to greater dilution of the injected material by the larger volume of blood remaining in the femoral tree. When the quantity of the injection was increased to 1.5 cc. in the case of the femoral artery and to 1.0 cc. in the case of the brachial, solutions of the same concentration sufficed to elicit a response. Further increase in the amount of solution injected did not lower the threshold for stimulation. It was concluded that the volumes named are sufficient to displace the blood from the respective arteries of a cat of average size and thereafter these quantities were used.

RESULTS. One can distinguish several types of response to the intra-arterial injection of chemical solutions of various sorts. Certain solutions stimulate neurones subserving pain-sensibility. In anesthetized animals this results in an ummistakable pseudaffective reaction, characterized by

movements of the extremities, hyperpnea, and, in many instances, vocalization. The intensity of reaction varies depending upon the strength of stimulation and the degree of anesthesia. Reflex activity usually occurs in all four extremities. However, in many instances, the more active motions are seen in the extremity which has received the solution.

This reflex activity is so marked in most cases as to obscure a local reaction confined to the tissues which receive the injected material. The local response is best seen after all afferent fibers which may initiate reflex activity have been interrupted. In the denervated hind limb it consists of a slow and sustained extension and stiffening of the extremity and in the denervated fore leg of a gradual flexion of the paw at the wrist-joint. Apparently it results from a direct action of the irritant upon local structures.

A third type of reaction is a local effect which follows the injection of a larger quantity of a hypotonic solution. If 3 cc. of water are injected into a brachial or femoral artery there occurs a rapid twitching of the musculature of that extremity. The response is usually confined to the limb injected but in certain cases the twitching is prolonged and becomes sufficiently violent to give reflex signs of painful stimulation. The twitching is not abolished by denervation of the limb and hence must be local in origin. It does not occur if the solution injected has a sodium-chloride content exceeding 0.3 per cent, i.e., a molar concentration of ions in excess of 0.09.

Of the three types of response described only the first has been considered indicative of the stimulation of nerve-endings subserving pain-sensibility. The occurrence of this pseudaffective reaction following intra-arterial injections was made the criterion of such stimulation.

Acid solutions. Since dilute acids stimulate the pain-receptors in question (Moore and Moore, 1933) experiments were performed to determine the hydrogen-ion concentration required for this effect. Solutions of known pH values were prepared by combining varying quantities of decinormal acetic acid and of decinormal sodium hydroxide according to the data of Cohn, Heyroth and Menkin (1928). Solutions having a pH of 6.0 provided stimulation in animals under anesthesia of moderate depth. In several animals in which anesthesia was deep pseudaffective responses were not observed until a pH as low as 5.7 was attained This variation suggests that in unanesthetized animals sodium-acetate solutions having a pH even greater than 6.0 might suffice to stimulate. Since, as will be shown below, neither sodium ion nor acetate ion is irritating in decinormal concentration, such stimulation is necessarily a function of the acidity of the solution.

Alkaline solutions. To determine the concentration of hydroxyl ion necessary to produce stimulation Sørensen's table of borate mixtures

(1909) was used, combining varying quantities of decinormal sodium borate and of decinormal hydrochloric acid. The pH values of these solutions were corroborated by means of the indicator *thymol blue*. Decinormal sodium borate, having a pH of about 9.2, produced unmistakable signs of stimulation. Even slight neutralization with acid, to pH 9.0, abolished this effect. Since it was found that neither sodium ion nor borate ion is irritating in decinormal concentration, the stimulus resulted from the alkalinity of the solution.

Solutions of neutral salts. The concentrations at which numerous salts produce stimulation are shown in table 1. Since it is not certain which expression is the more significant, each value is given both in terms of concentration of the salt and in terms of ionic concentration. To avoid confusion with the effects of hydrogen ion and of hydroxyl ion, only solutions within the range pH 6.5 to 8.0 were used. As shown in table 1 hypotonic solutions were rendered isotonic by addition of sodium chloride. All ions of the Hofmeister series (Loeb, 1924) were tested except the ammonium ion and the tartrate ion which formed solutions having pH values outside the limits imposed.

For each salt, trials were made first with a solution too dilute to produce stimulation. Then the concentration was increased by increments of 1 gram of solute per 100 cc. solution until an irritating strength was reached. When the effective concentration was less than a 1.0 per cent solution the determination was made to an accuracy of 0.25 gram solute per 100 cc. of solution. Each determination was repeated in at least four animals.

The degree of dissociation of each salt at its effective concentration was determined by means of data upon conductances of electrolytic solutions obtained from Kraus (1922) or the International Critical Tables (1929).

Discussion. Additional experiments have led to the belief that the pain-fibers which are stimulated by the intra-arterial injection of non-corrosive but irritating solutions end in or about the smaller vascular branches (Moore and Moore, 1933). Solutions which provide stimulation when injected peripherally into an artery do not produce visible reflex effects when injected centrally into the accompanying vein. Hence the irritation occurs before the solution has been returned to the general circulation. Moreover, the nerve-endings of cutaneous sensibility are not essential. The solutions used may evoke quite intense reactions when injected into an artery of purely visceral distribution such as the hepatic. The pain-receptors in question must therefore be located either in the blood-vessels or in the peripheral tissues which the vessels supply.

Burget and Livingston (1931) found that 5 per cent lactic acid elicited signs of painful stimulation when introduced by way of the brachial artery but produced no visible reflex effects when injected directly into the tissues of a limb. We have confirmed this observation repeatedly. Solutions of

moderate concentration provide stimulation when injected into the artery but produce no noticeable effect when injected directly into the tissue which the artery supplies. Similarly, although the injection of a highly concentrated solution directly into the liver substance has occasionally produced a slight reaction, subsequent injection of the same solution into the hepatic artery elicited a more immediate and much more striking response. These facts have led to the conclusion that the endings affected are situated in close association with the vascular tree.

In the previous communication (Moore and Moore, loc. cit.) experiments were described in which irritating solutions failed to produce painful stimulation when they were confined to the large arteries by ligation or embolic obstruction of the smaller vessels. During the present study it was noted that stimulation occurred only when conditions permitted the stimulating agent to reach the capillary bed in an effective strength. In a cat of average size a decrease in the volume of injection below 1.5 cc. for the femoral artery or 1.0 cc. for the brachial necessitated an increase in concentration if an effect was to be obtained. In some cases, following arterial ligation, the venous trunk of the extremity was ligated and the solution injected peripherally into the vein. Under such conditions the usual threshold concentrations were not effective until a volume was injected which exceeded that required when the smaller arterial trunk was used. Moreover, with careful injection, one sometimes observed a column of colorless reagent slowly displacing a column of blood from the arterial or venous stem. It could be seen that stimulation did not occur while the injected material was confined to the vascular trunks. These facts are reasonably explained if one assumes that the pain-endings have a peripheral location and that the injected materials suffer dilution by the residual blood. Whether the receptors are in the capillaries or about them their location is such as to expose them to chemical effects which may arise from abnormal conditions in the tissues which the vessels supply.

That, in anesthetized animals, these endings are stimulated by hydrogen ion in a concentration as low as that represented by pH 6.0 is of interest in relation to the pain which uniformly accompanies ischemic conditions. Lewis, Pickering and Rothschild (1932) concluded that in ischemic contraction of skeletal muscle pain results from a stimulus which is derived from the accumulation of some factor as a result of physiological processes. Meyerhof and Lohmann (1926) have shown that frog muscle may attain a pH as low as 5.6 upon complete fatigue. The muscles of man become completely fatigued in intermittent claudication and acute pain characterizes the process. Keefer and Resnik (1928) have presented evidence to support the view that anginal pain results from myocardial anoxemia, while Dawson and Bodansky (1931) have demonstrated that perfusion of the mammalian heart may be interrupted for so long a period as to

render subsequent washings as acid as pH 5.3. It is our belief that in ischemic or gangrenous processes the accumulation of the acid products of anoxidative metabolism in areas virtually devoid of circulation is of sufficient magnitude to provide a stimulus to the pain-endings we have described.

On the alkaline side of neutrality stimulation did not occur until a pH of 9.2 was reached. In view of this fact it is highly improbable that visceral pain ever results from an accumulation of hydroxyl ion.

TABLE 1

Minimum concentrations at which various salts stimulate the pain-receptors described

REAGENT	MOLAR CONCENTRATION REQUIRED FOR STIMULATION				REAGENT	MOLAR CONCENTRATION REQUIRED FOR STIMULATION			
	Salt	Cation	Anion	Total Ions		Salt	Cation	Anion	Total Ions
Chlorides					*Sodium salts*—Contd.				
Sodium chloride	0.51	0.39	0.39	0.79	Sodium thiocyanate**	0.33	0.24	0.24	0.48
Lithium chloride	0.47	0.36	0.36	0.72	Sodium acetate	0.33	0.23	0.23	0.46
Caesium chloride	0.45	0.35	0.35	0.70	Sodium sulphate	0.32	0.33	0.16	0.49
Calcium chloride	0.41	0.28	0.55	0.83	Sodium thiosulphate†	0.28			
Magnesium chloride	0.295	0.20	0.40	0.60	Sodium lactate†	0.17			
Strontium chloride	0.19	0.12	0.24	0.36	Sodium fluoride	0.12	0.10	0.10	0.20
Potassium chloride	0.13	0.11	0.11	0.22	Sodium phosphate‡	0.14	0.27	0.09	0.36
Rubidium chloride*	0.08	0.07	0.07	0.14	Sodium citrate**	0.06	0.11	0.04	0.14
Barium chloride*	0.02	0.02	0.03	0.05	*Potassium salts*				
Sodium salts					Potassium chloride	0.13	0.11	0.11	0.22
Sodium chloride	0.51	0.39	0.39	0.79	Potassium nitrate*	0.10	0.08	0.08	0.16
Sodium bromide	0.39	0.31	0.31	0.62	Potassium bromide*	0.04	0.04	0.04	0.07
Sodium iodide**	0.33	0.27	0.27	0.53	Potassium iodide*	0.03	0.03	0.03	0.05
Sodium nitrate	0.33	0.25	0.25	0.50					

* In solution in 0.7 per cent NaCl having an ionic concentration of 0.20 mole not included in table 1.

** Used dissociation value for corresponding potassium salt.

† Data upon the degree of dissociation not available in standard works.

‡ A neutral mixture of monobasic and dibasic salts.

The threshold concentrations at which chlorides of the alkali metals and of the alkaline earths provide stimulation are shown in table 1. Ammonium and beryllium chlorides were not tested inasmuch as they form acid solutions. The chlorides of sodium, lithium, caesium and calcium were not effective until 0.7 to 0.8 molar concentration of ions was reached. It is obvious from the results tabulated for calcium chloride and for sodium chloride that the stimulation was not an effect of the chloride ion. In view of the similarity of the ionic-concentration figures in the four instances

named it seems probable that these values represent a concentration at which solutions exert a marked osmotic effect upon nerve-endings. Inasmuch as the same anion was used throughout the series the results give a rough measure of the irritating effect of the various cations. In order of increasing power to stimulate the pain-receptors they may be arranged as follows: 1, sodium, lithium and caesium; 2, calcium; 3, magnesium; 4, strontium; 5, potassium; 6, rubidium, and 7, barium. In this series the power to stimulate pain-endings apparently bears no relation to atomic weight or to the order of the Hofmeister series (Loeb, 1924).

In like manner various sodium salts were tested to determine the relative power of various anions to provide stimulation. The results indicate that the chloride ion is least irritating of all. The other ions range in the order listed in table 1, the citrate ion being most effective. Again, the power to provide stimulation apparently bears no relation to atomic weight or to the Hofmeister series. The order of the first five anions named corresponds to their decreasing ability to depress the osmotic pressure of egg albumin as determined by Lillie (Höber, 1922), but Lillie found the sulphate ion to precede the chloride ion in such a list.

The third series of table 1 illustrates the effect of combining a cation having a relatively high power to stimulate (potassium) with an anion of similar type (bromide, iodide, nitrate). There is apparently a summation of effect as a result of which stimulation occurs at a lower concentration than is required when either ion acts alone.

Another interesting observation relates to the influence of dilute solutions. Rubidium chloride, barium chloride, sodium citrate, potassium bromide, potassium iodide and potassium nitrate were sufficiently irritating to provide stimulation in hypotonic concentrations. When these solutions were rendered isotonic by addition of sodium chloride the threshold concentration for stimulation was raised. For example, an aqueous solution of 0.1 per cent barium chloride ($BaCl_2 \cdot 2H_2O$) sufficed to stimulate but when the same salt was dissolved in 0.7 per cent sodium chloride a concentration of 0.5 per cent was required. Apparently, although hypotonic solutions of non-irritating ions fail to stimulate, the action of irritating ions is enhanced by low salt concentration. This suggests that the irritability of the nerve-endings may be increased when they are bathed in a hypotonic solution.

During the past few years there have been several inquiries into the part which chemical changes may play in producing the pain of inflammation. The work of von Gaza and Brandi and that of Schade has been reviewed by Häbler and Hummel (1928) and by Reimers (1932). These authors conclude that the pain of inflammation and of inflammatory exudates arises in part from 1, increase in the content of potassium ion; 2, increase in local acidity, and 3, increase in local osmotic pressure. It is interesting

to note that the pain-receptors with which we have been concerned are highly sensitive to potassium ion (table 1), especially if it is associated in solution with other ions possessing some degree of irritant power. Moreover, these pain-endings are stimulated by a concentration of hydrogen ion considerably lower than that which, according to Häbler (1927), regularly occurs in acute inflammatory pus. On the other hand, the values given in table 1 for sodium chloride and for lithium chloride indicate that a very great increase in salt concentration must occur in order for pain to arise as a result of the osmotic pressure.

Although the pain-receptors of the skin are sensitive to caustics they are not stimulated by chemical changes of the order employed in the experiments we have described. Pain which arises from the cutaneous surface accompanies gross traumata and is the result of destructive stimulation, be it mechanical, thermal or corrosive in form. In contrast, pain which arises from within the tissues is the result of factors which are poorly understood. Two common processes accompanied by such pain are inflammatory reaction and arterial insufficiency. It has been widely believed that in the former process pain results from the increased tension in the tissues. However, arterial insufficiency may be accompanied by acute pain without the occurrence of visible swelling and in various edematous conditions extreme tumefaction may arise rapidly without the occurrence of appreciable pain. Tissue processes which give rise to pain are accompanied regularly by marked changes in local physicochemical conditions. The experiments which have been reported indicate that pain-endings in proximity to the capillary loops are peculiarly sensitive to stimuli of a chemical kind. It is our belief that this group of receptors constitutes an important mechanism in the occurrence of visceral pain.

SUMMARY

The pain which follows the intra-arterial injection of various solutions arises from stimulation of afferent endings which are located in proximity to the capillary bed (see p. 596). Experiments have been performed to determine the chemical properties which serve to stimulate these pain-receptors in animals under amytal anesthesia.

Solutions as weakly acid as pH 6.0 provide stimulation. Solutions as alkaline as pH 9.2 have a similar effect (pp. 595, 596). Solutions having a pH value between these limits do not produce pain unless they possess a molar ionic concentration of 0.7–0.8 or contain some substance which operates to exert an irritant action upon the pain-endings concerned (p. 598).

The minimum concentrations at which various neutral salts provide stimulation are listed in table 1. The results for the chlorides tested indicate that injections of sodium, lithium, caesium or calcium ions have

relatively little effect upon nerve-endings, whereas the ions of strontium, potassium, rubidium and barium are increasingly irritating in the order named. Of the anions tested the chloride is least effective. Other anions range in the order listed (table 1, sodium salts), the citrate ion possessing the greatest irritating power. The ability of ions to stimulate pain-receptors apparently bears no relation to atomic weight or to the order of the Hofmeister series. Whether the stimulation represents a specific action of the ion upon the nerve-ending or is secondary to a disturbed ionic equilibrium is not known.

When two irritating ions are combined in solution there is a summation of effect as a result of which stimulation occurs at a lower concentration than is required when either ion acts alone (table 1, potassium salts).

Evidence is presented which indicates that the threshold for stimulation is lowered when the receptors are exposed to hypotonic solutions (p. 599).

The relation of these observations to current ideas of pain-production in inflammation is discussed (p. 599) It is suggested that chemical changes, e.g., the accumulation of acids or of potassium ion in inflamed areas, may be of more importance than increased tissue-tension.

The pain which accompanies ischemia is noted (p. 597). It is believed that such pain results from local accumulation of acid metabolites.

Tissue processes which give rise to pain are accompanied regularly by marked changes in local physicochemical conditions. The receptors which we have described are peculiarly sensitive to chemical stimuli and may constitute an important mechanism in the occurrence of visceral pain.

It is a pleasure to express our indebtedness to Dr. B. M. Hendrix and to Dr. Meyer Bodansky for their helpful criticism and advice during the progress of our experiments.

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ON THE LYSIN PRESENT IN NORMAL URINE

JULIUS C. ABELS

From Washington Square College, New York University

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The presence of an hemolysin in human urine was first described by McKee (1915), who found a lysin in about 30 per cent of urines, and who thought that its appearance had some pathological significance. Ponder later showed that the lysin is present in over 90 per cent of normal human urines, and that although it may be absent on any one day, it appears from time to time in all normal individuals (Ponder, 1922a, b.) Miller Lamb confirmed this in 1927. Ponder found the lysin to be heat stable, alcohol soluble, and inhibited by plasma; these and other considerations led him to suggest that the lytic substance is one of the bile salts. Miller Lamb, however, was unable to find a parallelism between the lytic activity of urine and the quantity of bile salts contained in it, but he found a very striking parallelism between the lytic activity and the surface tension of the urine, the most lytic urines showing the least surface tension. He accordingly concluded that the lysin is a surface active substance, although not one of the bile salts. (Miller Lamb, 1927.)

This paper is concerned with a re-investigation of the subject, and with a further attempt to identify the lysin.

Methods. The technique employed for measuring the lytic activity of the urine is essentially that described by Ponder. A red cell suspension is prepared, usually from human blood, the thrice washed cells of 1 cc. of blood being finally suspended in 100 cc. of 0.9 per cent NaCl. (Kahlbaum.) To each of a series of tubes containing 0.45 cc., 0.36 cc. . . . . 0.06 cc. of the urine is added 1 cc. of this suspension, and the tubes are kept at 37° for 4 hours. The lytic activity of the urine is then expressed as the quantity which produces complete lysis at the end of this time.

All the urines examined were morning specimens, tested within a few hours of their being passed. About 50 per cent were derived from normal persons, and the remainder from patients in hospitals, but it may be remarked at once that the appearance of the lysin, or the lytic activity of the urine, is not dependent on there being a pathological state.

Results. 1. *The occurrence of the lysin.* I have obtained substantially the same results as Ponder's and Miller Lamb's. In a total of 2026 urines examined, the lysin was found to be present in 93 per cent of the cases, and

absent in the remainder. In some cases the lytic activity was found to be great, and in others smaller, and the following table shows the percentage number of urines which were found to have various degrees of lytic activity.

TABLE 1

	ACTIVITY					
	Negative	0.45 cc.	0.36 cc.	0.30 cc.	0.16 cc.	0.06 cc.
Per cent of total	7	2	3	11	55	22

The total number of 2026 was made up by examining the urines of 72 persons for a period of between 2 and 5 weeks, the urines of 400 individuals for one day only. Although the urine of any one person may sometimes be non-lytic, I have never found it to be so for more than 3 or 4 days in succession, unless the urine was persistently alkaline (see below).

The lysin also occurs in normal urine from the rabbit, cat, rat, and dog. Non-lytic urines are quite often found in rabbits, but rarely in dogs and cats. The lysin accordingly appears to be of quite general occurrence, and it exhibits no specificity; rabbit urine, for instance, is lytic for the cells of man, the cat, and the dog, if it is lytic at all. There are nevertheless the usual differences in the resistance of the cells of the various animals to the lysin, the least resistant cells being those of man, then those of the rat, then those of the cat, and rabbit cells being most resistant of all. This is not the resistance series for either of the bile salts.

2. *The surface tension of lytic urines.* In view of Miller Lamb's statement that lytic urines usually show a low surface tension, I measured the surface tension of 400 urines by the Du Nouy tensiometer. Since the surface tension of urine decreases rapidly after it is passed, all the measurements were made within a few minutes after collection. Of the total number, 354 urines were lytic, and were found to be grouped with respect to their surface tensions in the following manner.

TABLE 2

	49–51 DYNES	51–53 DYNES	53–55 DYNES	55–57 DYNES	57–59 DYNES	59–61 DYNES	61–63 DYNES
Number	50	139	99	52	14	0	0

Most of the lytic urines are accordingly those of a low surface tension. For the 46 non-lytic urines, the distribution is as follows:

TABLE 3

	49–51 DYNES	51–53 DYNES	53–55 DYNES	55–57 DYNES	57–59 DYNES	59–61 DYNES	61–63 DYNES
Number	0	5	8	13	17	2	1

The surface tension of the non-lytic urines thus appears to be distinctly higher than that of the lytic ones. The relation between surface tension and lytic activity is, however, less than appears at first sight, for if we take the 354 lytic urines, and correlate their activity, as quantitatively measured, against their surface tensions, we get a correlation coefficient of only −0.42. Quite frequently, one gets a very lytic urine with a high surface tension, and sometimes a non-lytic urine shows quite a low tension. Correlating total solids with lytic activity gives a coefficient of 0.64, which is much more significant, and it is very likely that the lytic activity is determined principally by the extent to which the urine is concentrated, and that the lower tensions found in the more lytic urines merely indicate that these urines are the more concentrated. Large quantities of lysin are, of course, likely to be present in concentrated urines.

Miller Lamb's conclusion that there is a close relation between lytic activity and surface tension is accordingly not confirmed. Such relation as exists is probably due to lytic activity and surface tension being affected by a common factor, viz., the extent to which the urine is concentrated.

3. *The relation of the lytic activity to the bile salt content.* I have been unable to find any clear relation between lytic activity of urine and the amount of contained bile salts. Using Oliver's, Petenkoffer's, and Udransky's tests on 280 lytic urines, I found that in the great majority of cases (about 95 per cent) the tests for bile acids were positive, but that in some few cases they were negative. Similarly, in 30 non-lytic urines, bile acids were present in about 95 per cent of cases, and absent in the remainder. This confirms Miller Lamb's observation.

Although bile salts (or acids) are present in most non-lytic urines, and occasionally absent, so far as the tests show, in lytic ones, these facts alone do not altogether exclude the possibility that the lysin is one of the bile salts. I shall show below that urines with a pH greater than about 6.2 are invariably non-lytic, and so the fact that urines containing considerable quantities of bile salts are non-lytic may be accounted for by supposing that the lysin is reduced in activity as the pH increases, which, indeed, is quite characteristic of lysins of the bile salt class (Gordon, 1933). Similarly, the occurrence of lysis in urines which do not contain sufficient bile salts to be detected by the above tests may be due to there being a small quantity present, and to the activity of this small quantity being greatly increased by the presence of other substances present in the urine.

4. *The effect of pH.* Urines of a pH less than 5.6 are almost invariably lytic, and those of a pH greater than 6.2 are invariably non-lytic, unless they are extremely ammoniacal. Table 4 shows the results obtained in 350 cases, the pH being determined by the quinhydrone electrode.

TABLE 4

	pH 5.3	pH 5.4	pH 5.5	pH 5.6	pH 5.7	pH 5.8	pH 5.9	pH 6.0	pH 6.1	pH 6.2
Number lytic	10	31	68	80	50	39	6	1	1	0
Number non-lytic	0	0	0	4	8	7	9	12	12	12

Urines of a pH of less than 5.2 were discarded, as lysis might occur from the pH of such urines alone (Cole, 1915). The urines were all tested within ten minutes of being passed, for there is a decrease in pH on standing for a period of two or three hours, and this is accompanied by an increased lytic activity, as might be expected. As also might be expected, the urines of persons on a wholly vegetarian diet are generally non-lytic (80 per cent non-lytic urines obtained in 100 cases), for the urines of such persons are generally alkaline.

5. *Attempts to identify the lysin.* 1. As stated by Ponder, the lytic activity of urine does not appear to be related to the quantity of any ordinary constituent contained therein. I have satisfied myself on this point by determining chlorides, phosphates (inorganic, non-conjugated, and total) ammonia, total N_2, urea, uric acid, hippuric acid, oxalates, creatin, creatinin, total amino-acids, and sulphates. These determinations were carried out quantitatively by standard methods, and qualitative determinations were carried out for the following substances in addition: leucine, tyrosine, aromatic oxy-acids, skatol, phenol, cresol, indol, cystin, and indican. Uric acid and inorganic phosphates were definitely related to the pH of the urine, and since the lytic activity is dependent on pH too, these two urinary constituents may play an indirect part in determining the total lytic activity.

An even more certain way of showing that the hemolysin is not one of the ordinary constituents of urine is to show that no ordinary constituent is itself an hemolysin. This I have done by testing the lytic effect of each one of the common constituents separately in the concentration in which they may be expected to occur in urine, and also by preparing synthetic urine which, like the solutions of the common substances, is non-lytic.

2. By extracting the residue from evaporated urine with various solvents, it can be shown that the hemolysin is insoluble in carbon tetrachloride, chloroform, carbon disulphide, ethyl ether, acetone, benzene, toluene,

toluol, methyl, propyl, butyl, amyl, and capryllic alcohols. In carrying out such tests, it is very important to free the final preparation from all traces of the solvents, for many of the solvents are themselves lytic. In ethyl alcohol, on the other hand, the lysin is soluble. These conclusions as regards solubility confirm the conclusions of Ponder.

3. The lysin is remarkably heat stable, in that urine can be boiled for as long as an hour without its lytic activity being reduced. Boiling for two hours, however, results in permanent inactivation, as also does heating to 120°C. for 20 minutes. Exposure to higher temperatures results in an almost immediate inactivation. Cooling to −60°C. also results in inactivation.

4. The lysin is not very readily diffusible through fishskin or collodion, but it is difficult to obtain definite results because the activity of the hemolysin tends to fall off with time, and therefore during the progress of a diffusion experiment. On the other hand, the lysin passes readily through a Berkefeld filter.

5. Indirect evidence of the lysin's being a surface active substance is provided by the fact that it can be adsorbed on "Darko" (an activated charcoal), and also partially on Permutit. I have made many attempts to extract the lysin from the Darko, by simple washing, by extraction with ethyl alcohol, with N/1000 HCl, and with N/1000 NaOH, but these attempts have altogether failed.

6. As has already been stated, highly lytic urines are almost invariably of a pH less than 5.6. This suggests that the hemolysin may be inactivated in solutions of a pH greater than this, and such indeed appears to be the case. By raising the pH of various lytic urines by means of the addition of NaOH, Na_2HPO_4, and $NaHCO_3$, the lytic activity can be shown to decrease as the pH increases, and it can also be shown that this effect is irreversible, i.e., that the lytic activity of a urine at pH 5.4, for instance, is destroyed by bringing the pH to 7.5, but is not again restored by re-acidifying to pH 5.4. This is a very important point, for the same is not true of solutions of most simple hemolysins, and particularly it is not true of the bile salts. This, indeed, is the only property of the lysin which is completely inconsistent with Ponder's suggestion that it is one of the bile salts or acids.

7. Like most simple hemolysins, the lytic substance in urine is inhibited by small quantities of serum or plasma. The effect is principally due to the contained proteins, for deproteinised plasma, although having a small inhibitory effect, is relatively ineffective (cf. Ponder, 1923). The small inhibitory effect of deproteinised plasma can be accounted for by the inhibitory effect of its contained lipoids. The lysin is inhibited by lecithin, and to a smaller extent by cholesterol, although the quantities of these substances necessary to bring about any given degree of inhibition are at

least ten times greater than the amounts contained in plasma. Since nearly all hemolysins are inhibited by protein, and to a lesser extent by the lipoids, these results give no indication as to the nature of the lysin in urine.

DISCUSSION. It is easier to exclude various substances as being the lysin of normal urine than to say what the lysin actually is. On the grounds of its irreversible destruction by a change of pH, we can exclude the bile salts and such lysins as the soaps and glucosides, and the fact that all the known constituents of urine mentioned in section 5, 1, above, are either non-lytic or not proportional in quantity to the lytic power of the urine allows us to exclude them as well. There remain two possibilities: *a*, that the lytic substance is one of the unidentified components of urine, or *b*, that it is a bacterial lysin, produced in the body, and excreted in the urine. If it is the latter, it must be a lysin produced by an organism which is normally non-pathogenic, and the organism which suggests itself is B. coli.

At first sight this may not appear very likely, but there are in fact many properties common to the hemolysin of B. coli and the unknown lysin of the urine. The solubilities of the two lysins are the same, as also is their diffusibility, their adsorbability, and the extent to which they are inhibited by proteins, lecithin, and cholesterol. The lysin of B. coli is remarkable for its heat stability, and in this respect is similar to the unknown lysin: both lysins, moreover, slowly disappear with the lapse of time, i.e., are unstable in a sense which lysins like saponin, etc., are not. The most important similarity lies in the lysin of B. coli and the unknown lysin both being irreversibly destroyed by pH changes; this, of course, is a common property of bacterial lysins, but taken together with the heat stability, points quite clearly to the possibility of the lysin's being a B. coli lysin. I have, in fact, been able to duplicate every known characteristic of the unknown lysin by using media containing the lysin of hemolytic strains of B. coli, and while it is of course impossible to prove the identity, the possibility of the identity remains.

SUMMARY

This paper is concerned with an attempt to identify the lysin present in normal human urine and also in the urine of normal animals. The lysin is remarkably heat stable, is soluble in ethyl alcohol but not in most organic solvents, is diffusible, adsorbed on activated charcoal, inhibited by plasma proteins, lecithin, and cholesterol, and is irreversibly destroyed at a pH of 8.0 to 9.0. It is not the bile acids or their salts, and its activity is much more highly correlated with the pH of the urine than with its surface tension. It may be one of the unidentified components of the urine, but its properties are very like those of certain bacterial hemolysins, and in particular like those of the lysin of B. coli.

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THE ACTION OF OXYGEN IN COUNTERACTING ALCOHOLIC INTOXICATION

ALVAN L. BARACH

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City

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Physiologists have frequently noted the similarity between the symptoms of alcoholic intoxication and oxygen-want, as encountered at high altitudes and in experiments in low oxygen chambers. In both states, there are characteristic loss of neuromuscular control, retarded mental processes, lack of self-criticism and a general releasing of inhibitions. After Yandell Henderson's observation that inhalation of 7 per cent carbon dioxide caused a rapid de-etherization after narcosis, Hunter and Mudd (1) observed that patients in coma, due to alcohol, were improved by the inhalation of increased concentrations of carbon dioxide. Palthe (2) reported that the symptoms of alcohol intoxication were largely relieved in human subjects and in animals by the inhalation of pure oxygen through a mask. He found that the inhalation of oxygen prevented death in rabbits who had been given alcohol in doses that were lethal to control animals.

The purpose of the present investigation was to determine whether alcohol could be considered an anoxemia-producing agent and, if so, to what extent. Himwich and his collaborators (3) found that the ingestion of alcohol by men and dogs increased the lactic acid content of the blood two-fold after two and a half hours. Previously, Katz and Long (4) demonstrated that oxygen-want, produced by lowering the oxygen concentration in the respired air below 8 per cent, resulted in an accumulation of lactic acid in the circulating blood, the skeletal muscle and the cardiac muscle, and that the mammalian heart muscle became exhausted when the lactic acid content increased over two and a half times the resting muscle content, whereas skeletal muscle withstood four times the normal amount.

If alcohol creates a state of oxygen deficiency in the tissues, this effect should be more clearly manifested in conditions in which an increased need for oxygen is present. Muscular exercise calls for an increased oxygen supply which is ordinarily met by increases in the pulmonary ventilation and the blood flow.

Quantitative measurements of these functions, such as pulse rate, respiratory rate and pulmonary ventilation, could therefore be expected to reveal any interference with oxidative processes induced by alcohol.

Observations were made on the influence of oxygen on normal subjects and on subjects after alcohol ingestion, at rest and after standard exercise, and on animals at rest.

METHODS. *Type of exercise.* The exercise consisted of mounting and descending a wooden platform eight inches high, forty times a minute. A metronome was used to regulate the frequency of step. The pulmonary ventilation was obtained by collecting the expired air in a Douglas bag for two minutes and measuring the volume by passing it through a gas meter.

The exercises were performed for 2, 4 and 8 minutes, with a ten minute rest period between each test. When air was inhaled during the experiments, a mouthpiece was also employed so as to conform to the experiments with oxygen. The alcohol was administered on an empty stomach, 60 cc. of 95 per cent alcohol diluted with one orange and one lemon and water to make a final dilution of 200 cc. The results were obtained on three individuals, seventeen tests on the exercises without alcohol and eight tests with alcohol. The averages of these determinations were charted, since no essential deviation in the individual results was encountered.

In the experiments in which alcohol was ingested, no food was taken previously, as the mixture of alcohol and fruit juices provided the equivalent of a breakfast, approximately 500 calories. In the tests without alcohol, the individual was allowed breakfast, which was the same in the air and oxygen experiments. The tests were repeated to obviate the factor of practice exercising a confusing influence.

RESULTS. The result of breathing oxygen in the standard exercise test on normal subjects showed a greater efficiency and ease of performance than when the subjects inhaled atmospheric air. In the three men tested, the decrease of pulse rate when inhaling oxygen as opposed to air was approximately 20 per cent, after the 2, 4 and 8 minute exercises. The respiratory rate was 10 per cent lower, and the pulmonary ventilation 13 per cent lower after the eight minute exercise. The detail of the comparative response is shown in table 1 A.

The effect of the ingestion of 60 cc. of alcohol diluted in 200 cc. of water before the standard exercise showed a marked decrease in efficiency. The maximum increase in the pulse rate over the control response without alcohol was 16 per cent, after the eight minute exercise; for the respiratory rate, 35 per cent, after the four minute exercise; for the pulmonary ventilation, 30 per cent, after the eight minute exercise (see table 2).

The result of breathing oxygen was now tested after the subjects had taken the same dose of alcohol. A marked improvement of efficiency was observed, as indicated by the decreased pulse rate, pulmonary ventilation and respiratory rate (see table 1 B). The effectiveness of oxygen in the alcoholic subject may now be compared to that of the subject without alcohol. The percentage reduction of pulse rate due to oxygen inhalation

TABLE 1

Effects of oxygen inhalation during exercise on men

A—Under basal conditions.
B—After alcohol ingestion.

		PERIOD OF EXERCISE, MINUTES	AVERAGE RATE IN AIR	AVERAGE RATE IN OXYGEN	PER CENT DECREASE BREATHING OXYGEN
A. Under basal conditions	Pulse	Rest	79.9	72.1	−9.8
		2	107.5	87.3	−18.8
		4	118.0	95.5	−19.1
		8	134.7	105.9	−21.4
	Respiratory rate	Rest	15.6	16.9	+8.3
		2	19.5	19.9	+2.1
		4	22.0	21.6	−1.8
		8	24.9	22.3	−10.4
	Pulmonary ventilation (liters per minute)	Rest	12.0	10.8	−10.0
		2	24.3	21.4	−11.9
		4	31.1	26.9	−13.5
		8	35.5	30.9	−13.0
B. After alcohol ingestion	Pulse	Rest	83.3	75.0	−10.0
		2	120.8	93.5	−22.6
		4	135.6	109.3	−19.4
		8	156.6	123.5	−21.1
	Respiratory rate	Rest	16.5	16.3	−1.2
		2	23.3	21.7	−6.9
		4	29.8	24.7	−17.1
		8	31.8	26.7	−16.0
	Pulmonary ventilation (liters per minute)	Rest	11.5	19.9	−13.9
		2	29.5	21.6	−26.8
		4	38.8	31.6	−18.5
		8	46.0	33.1	−28.0

TABLE 2

Effects of alcohol ingestion on normal men during exercise

	PERIOD OF EXERCISE, MINUTES	AVERAGE RATE IN AIR WITHOUT ALCOHOL	AVERAGE RATE IN AIR WITH ALCOHOL	PER CENT INCREASE WITH ALCOHOL
Pulse	Rest	79.9	83.3	+4.3
	2	107.5	120.8	+12.4
	4	118.0	135.6	+14.9
	8	134.7	156.6	+16.3
Respiratory rate	Rest	15.6	16.5	+5.8
	2	19.5	23.3	+19.5
	4	22.0	29.8	+35.5
	8	24.9	31.8	+27.7
Pulmonary ventilation (liters per minute)	Rest	12.0	11.5	−4.2
	2	24.3	29.5	+21.4
	4	31.1	38.8	+24.4
	8	35.5	46.0	+29.6

is not significantly different in the two groups (A and B in table 1), but the respiratory rate and the pulmonary ventilation were consistently more diminished in the tests with alcohol. Thus, the effect of oxygen in reducing the respiratory rate was 15 per cent greater in the subject who had alcohol than it was in the control test, after the four minute exercise; and the reduction of pulmonary ventilation, 15 per cent greater after the two and eight minute exercises.

To summarize, the inhalation of oxygen increases the efficiency of normal subjects performing muscular work. The ingestion of alcohol markedly diminishes the efficiency of the performance of muscular work, but this efficiency can be partially restored by inhalation of oxygen, and to a degree that is greater than the percentage improvement which oxygen exerts without alcohol. This suggests that oxygen to some extent offsets the impairment of neuromuscular activity that results from alcohol ingestion.

At rest, the effects of oxygen on alcohol intoxication were more variable. In a study of the psychological changes which oxygen exerts in alcoholic subjects, which the author conducted in collaboration with Dr. Ross McFarland (to be published), there was a consistent improvement in mental functioning in most instances. The fundamental sense reactions were more accurately performed and there was usually a definite increase in intelligence. However, the inhalation of 50 to 100 per cent oxygen did not cause a cessation of the majority of the symptoms of intoxication except in one instance. In this subject, a small amount of alcohol, 40 to 60 cc., was sufficient to cause symptoms of marked intoxication. The inhalation of pure oxygen, or 50 per cent oxygen, for seven minutes restored him to a comparatively normal coöperative state. Withdrawal of oxygen plunged him, in less than two minutes, back into the same state of severe intoxication. When he inhaled 7 to 10 per cent carbon dioxide in oxygen for ten minutes, his mind became clear, and remained clear after cessation of treatment.

In twelve other subjects, the response to oxygen inhalation was consistently much less striking. Accurately conducted psychological tests were required to demonstrate the improvement in mental functioning. That a definite effect was produced on the symptoms of intoxication was generally demonstrable but, except in one case, was not as marked as that which Palthe described as occurring uniformly.

Experiments on rabbits were conducted to determine whether the lack of neuromuscular control which rabbits exhibit so strikingly after alcohol ingestion could be modified by inhalation of oxygen concentrations of from 50 to 100 per cent. A large oxygen tent (5) was used in which six to ten rabbits were placed and a concentration of 80 per cent oxygen employed. The rabbits were given by stomach tube 2 to 8 cc. of alcohol per kilo, and carefully observed in twenty different experiments.

The effect of inhaling high concentrations of oxygen on the behavior of the rabbits, who showed varying degrees of intoxication, appeared negligible. The control animals did not, on the whole, behave differently. Furthermore, the inhalation of 5 to 7 per cent CO_2 in oxygen, beyond causing hyperpnea, did not favorably influence the symptoms of intoxication. Lethal doses of alcohol caused the same percentage of deaths in the animals kept overnight in the oxygen tent as occurred to the animals not treated by oxygen. In these results, our experience is distinctly contradictory to that of Palthe.

However, we conducted an experiment which showed that oxygen inhalation under certain circumstances did play a rôle in combating alcohol intoxication. Rabbits, one-half hour after ingestion of 3 to 4 cc. of alcohol per kilo, show marked signs of impaired neuromuscular control, such as swaying or losing their balance when they walk. When such alcohol-treated rabbits were placed in an atmosphere of 9 per cent oxygen, they promptly (within two to three minutes) fell on their sides and seemed completely unable to move. Raising the oxygen concentration to that of normal air revived them slowly, within fifteen minutes; increasing the concentration to 75 per cent restored them to their previous state somewhat more quickly, approximately ten minutes. Lowering the oxygen concentration to 9 per cent again caused them to fall completely powerless on their sides.

Thus, when the rabbits who were treated with alcohol were exposed to 9 per cent oxygen, their intoxication changed from deficient muscular control to collapse. Power, although not coördination, returned to their legs when the oxygen concentration of the air was raised to that of atmospheric air, or above that. Rabbits who were not previously given alcohol never collapsed on exposure to 9 per cent oxygen.

SUMMARY

1. The effect of inhaling oxygen on a standard exercise test was to increase the efficiency of performance, as indicated by decreased pulse and respiratory rates, and decreased pulmonary ventilation. The ingestion of alcohol before the tests markedly diminished the efficiency of the work performed, as shown by increases in the same indices.

2. When oxygen was breathed after alcohol ingestion, there was a marked improvement in the performance of muscular work, as contrasted with the same test in air. The percentage reduction of the respiratory rate and pulmonary ventilation was greater than the improvement exerted by oxygen in the exercise without alcohol. This circumstance, coupled with the evidence above, suggested that the inhalation of oxygen counteracted to some extent the effects of alcohol.

3. The intoxication of rabbits was not appreciably influenced by breath-

ing air containing 50 to 90 per cent oxygen concentrations. Lethal doses of alcohol to rabbits caused an equal number of deaths to animals in air and to those kept in an oxygen tent. The inhalation of CO_2 in oxygen did not restore alcohol-treated rabbits to an unintoxicated state.

4. The influence of oxygen on alcoholic intoxication of men at rest was generally to improve their sensory functions and to increase their intelligence. In collaboration with Dr. Ross McFarland, accurately controlled psychological tests showed, in most instances, a definite betterment of the higher mental functions, such as memory and judgment, as well as sense perception. In one individual, oxygen inhalation almost completely relieved the symptoms of alcoholism. Cessation of oxygen treatment resulted in a prompt return of intoxication. However, the inhalation of 7 to 10 per cent carbon dioxide in oxygen was followed by a permanent improvement.

5. Animals who became intoxicated by alcohol, so that their movements were noticeably poorly controlled, collapsed when exposed to an atmosphere of 9 per cent oxygen. Rabbits untreated with alcohol did not show this response.

6. In conclusion, the rôle of oxygen in combatting the effects of alcohol intoxication appeared most noticeable when there was the greatest need for oxygen: 1, when severe muscular work caused a demand for large amounts of oxygen, as shown in the exercise tests in man; 2, when the oxygen concentration of the air breathed was lowered below normal, as in the animal experiments. In both instances, inhalation of oxygen was attended by noticeable betterment in their state. With men at rest, the need for accurate psychological tests to determine improvement and, in animals, the failure to modify gross drunkenness, showed that the intoxication produced by alcohol was not essentially of anoxemic origin, or, to state it more precisely, was not due to the kind of oxygen-want that is capable of relief by oxygen inhalation.

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AN ANALYSIS OF THE ANEMIA OF PREGNANCY IN THE RAT[1]

EVELYN C. VAN DONK,[2] H. FELDMAN AND H. STEENBOCK

From The Laboratory of Agricultural Chemistry, University of Wisconsin, Madison

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For some time a keen interest has been shown by clinicians in the study of the so-called physiologic anemia of pregnancy, because of its widespread occurrence (1, 2, 3, 4) and its relation to practical problems of therapeusis. In spite of this, numerous questions remain unanswered, e.g., *a*, is it a true anemia or is it an hydremia (5, 6)? *b*, Is it of dietary origin (4, 6) or is it the result of toxins (1)? *c*, Is it limited in occurrence to the human species or does it occur in mammals in general?

A serious drawback to a proper evaluation of even the very recent work has been the lack of accurately controlled data such as can only be obtained with animals. Some data have been reported from work with pregnant dogs (7, 8), cows and sheep (9, 10), but they are of uncertain value because they belong to a time of crude hematologic methods.

We became interested in the anemia of pregnancy in the rat in 1930 at which time we were studying the dietary effect of onions upon hemoglobin synthesis. We failed to produce an anemia by feeding onions, but noted that quite uniformly there occurred a reduction in the amount of hemoglobin as pregnancy became advanced. Previous to this an anemia of pregnancy in the rat had been reported by Scott (11), and by Sure, Kik, and Walker (12). Scott expressed himself as follows:

> The blood volume of pregnant rats is of the same order relative to their *gross* body weight (including fetuses) as that of non-pregnant rats.
>
> The hemoglobin of pregnant rats expressed in grams per gram *net* body weight (excluding fetuses) is diminished as compared with that of non-pregnant rats.
>
> Thus the hypothesis that diminution in hemoglobin percentage during pregnancy in rats is brought about by dilution of the blood is insufficient to account for the facts and we must postulate in addition a diminished formation or an increased destruction of hemoglobin in these animals during pregnancy.

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[2] E. R. Squibb & Sons fellow.

Sure and co-workers observed an anemia of pregnancy in rats kept on their stock diet. With rats on a synthetic diet containing an abundance of wheat germ and yeast they found no anemia.

In the present paper we shall report data on variations in the analysis of blood of rats on various diets and during the prepartum and post-partum periods with particular reference to the rôle of hydration.

EXPERIMENTAL. First of all we made a survey of the amount of hemoglobin in rats from our stock colony. These are kept on a ration of yellow corn 71.5, oil meal 15, crude casein 5, alfalfa meal 2, butter fat 5, bone ash 1, sodium chloride 0.5, with distilled water and fresh whole milk. In addition once a week the entire colony is given distilled water to which has been added for each pailful of 8 quarts 25 cc. of a 0.15 per cent KI solution.

Hemoglobin determinations were made from time to time on young virgin females placed in cages with young males. In some cases the time of expected birth of young was calculated from the time of mating as determined by vaginal smears (13); in other cases, the easier method of making determinations on all females periodically from the time that they were placed in cages with males was followed. In this case for purposes of calculation the period of pregnancy was assumed to be 21 days.

To a second group of rats we fed a modified stock diet enriched with iron and copper salts to determine the effect of an increased supply of these elements. We evaporated at frequent intervals a solution containing copper sulfate and ferric chloride, equivalent to 5.0 mgm. iron and 0.25 mgm. Cu, on 6.66 grams of the solid constituents of our stock ration. When this was dry it was mixed with one-half its weight of whole milk powder. By using milk powder in the ration instead of feeding fresh whole milk ad libitum the females were prevented from consuming an inordinate amount of milk which is intrinsically low in copper and iron.

For a third group of rats we compounded a special ration which we assumed would meet any probable dietary requirements for hemoglobin synthesis. This was composed of two parts. Part I consisted of wheat 25, yellow corn 25, oil meal 7, alfalfa 2, yeast[3] 5, dried beef liver 7.5, bone ash 1, and sodium chloride 1. This was kept mixed in bulk. At frequent intervals this was mixed in the foregoing proportions with part II. Part II consisted of fresh egg yolk 5, cod liver oil 1, wheat germ oil 1, whole milk powder 20, and 5 cc. of a mineral solution. This mineral solution contained in each cubic centimeter 5 mgm. iron, 0.25 mgm. copper, 0.25 mgm. manganese, 0.005 mgm. iodine and 0.005 mgm. arsenic. When added in these proportions this mineral solution represented the additional amounts of these elements which a rat received with the consumption of 20 grams of ration.

[3] From Northwestern Yeast Company, Chicago.

The hemoglobin values resulting from the use of these rations are shown in chart 1 and table 1. It will be noted that anemia developed progressively on all rations with the progress of pregnancy and approximately to an equal degree of severity irrespective of the supplements. It was most severe at parturition, undoubtedly because of hemorrhage. As the amount of hemorrhage is variable, it was obvious that dietary comparisons for this period were no longer possible. After parturition there usually followed a rapid recovery from the anemia but some individuals did not regain their former hemoglobin level even after 4 weeks.

When our experiments were almost completed we were informed of similar experiments having been completed by Dr. Helen S. Mitchell of the Battle Creek Sanatorium. We accordingly postponed publication until her results appeared in print (14). From them it is evident that we confirm her experience in failing to secure correction of the anemia by dietary additions of copper, iron, manganese, and yeast. In addition, we failed to get results with supplements of iodine, arsenic, dried beef liver, fresh egg yolk and cod liver oil.

It now appeared very improbable to us that the anemia of pregnancy observed in the rat was of dietary origin; and we therefore gave consideration to the possibility of blood dilution. This had already been suggested for the human type by Bland and Goldstein (15) and others and had been accepted as a possibility for the rat by Mitchell and Miller (14). Sure, Kik and Walker (12) had actually found a concomitant decrease in serum proteins.

For our studies we used young female rats which weighed about 200 grams at the beginning of the experiment. They were kept on screens in groups of 5 or 6, and each group was mated with a vigorous young male. They were given greens twice weekly in addition to their stock ration. The time of coitus was established by examinations for sperm in the vaginal smear (13). As mating in the rat is almost always followed by conception, and as the period of gestation in our colony is $21\frac{1}{2}$ to 22 days, we felt justified in using an animal on any calculated day of gestation with a fair assurance that an error not greater than 12 hours was introduced. It would have been preferable, of course, to have followed each rat in its development of anemia from day to day, instead of using individual rats for the analysis, but the size of the blood samples necessary for our analyses rendered such a procedure inadvisable.

Our hematologic study included the following determinations: hemoglobin, red cell counts, red cell volume by hematocrit, refractive index of the plasma, percentage of water in the whole blood, sedimentation index, and reticulocyte count. Standard values were established by averaging a number of readings taken on normal animals. The aforementioned determinations were made for the most part on one sample of blood drawn from the heart.

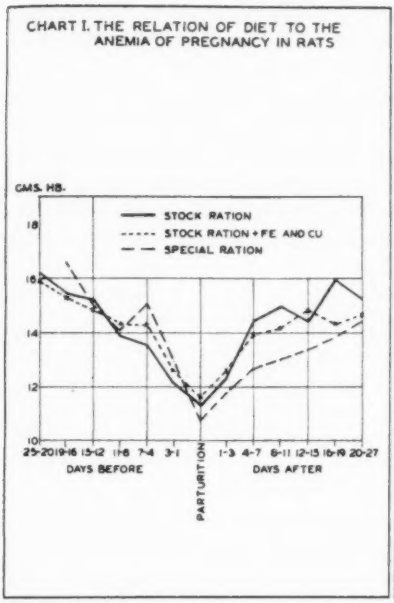
CHART I. THE RELATION OF DIET TO THE ANEMIA OF PREGNANCY IN RATS
GMS. HB.
STOCK RATION
STOCK RATION + FE AND CU
SPECIAL RATION
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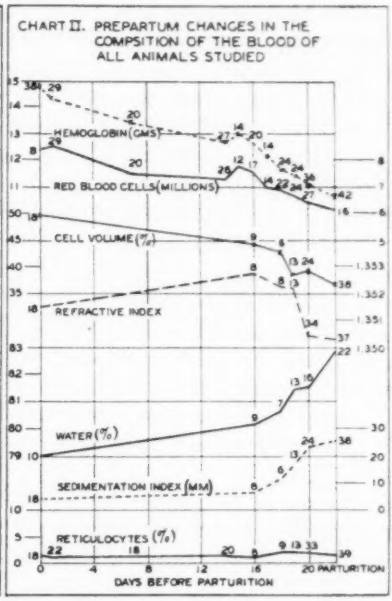
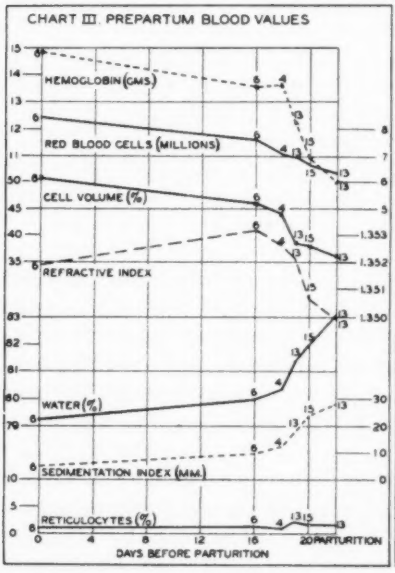
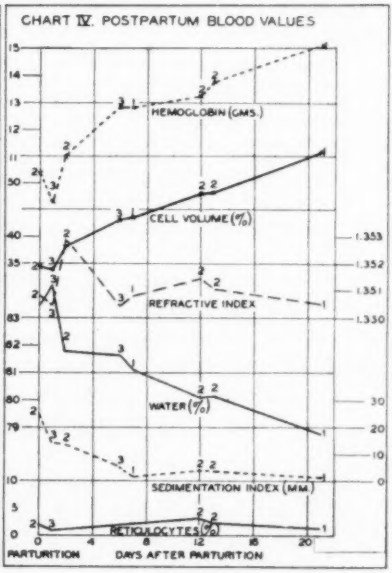
DAYS BEFORE
PARTURITION
DAYS AFTER







Numerals above curves represent the number of determinations made. Chart II shows average curves of the prepartum changes in the composition of blood of all the animals studied. The largest number of determinations was made on the days immediately previous to parturition since in our previous work the most significant changes were found to occur at this time.

The blood samples were obtained in the following manner: The animal was lightly anesthetized with 1 to 1.5 cc. of anesthetic ether[4] and then placed on an animal board. The forelegs were tied loosely to avoid stasis, and the cardiac apex was located by palpation. The fur over the heart area was brushed aside with a piece of cotton moistened with 95 per cent alcohol and a no. 26 gauge $\frac{5}{8}$-inch needle attached to a Luer syringe was plunged quickly into the heart. By gentle suction about 3 to 4 cc. of blood were drawn into the syringe without difficulty, after which the needle was withdrawn quickly. This procedure ordinarily took about 2 to 3 minutes.

TABLE 1

The relation of diet to the anemia of pregnancy in rats

INTERVALS	STOCK RATION		STOCK RATION + Fe AND Cu		SPECIAL STOCK RATION	
	Range in Hb	Average Hb	Range in Hb	Average Hb	Range in Hb	Average Hb
Before parturition						
25th-20th day	14.87–18.13 (20)*	16.25	13.65–17.31 (8)*	15.94		
19th-16th day	13.99–16.81 (7)	15.37			16.57 (1)	16.57
15th-12th day	12.75–16.57 (14)	15.18	12.48–16.33 (10)	14.82	14.99–15.25 (3)	14.93
11th- 8th day	10.45–16.10 (13)	13.99	13.65–14.99 (4)	14.19	13.20–15.06 (3)	14.19
7th- 4th day	10.94–15.88 (27)	13.56	10.45–16.10 (14)	14.21	12.75–16.81 (6)	14.99
3rd- 1st day	10.17–13.99 (23)	12.12	8.08–15.25 (18)	12.54	11.49–14.32 (7)	12.94
Parturition	6.55–13.20 (29)	11.37	3.48–13.46 (12)	11.44	8.08–12.90 (5)	10.73
After parturition						
1st- 3rd day	7.63–15.46 (9)	12.42	7.28–13.99 (7)	12.41	10.65–13.49 (3)	11.73
4th- 7th day	10.75–15.88 (27)	14.34	12.75–15.46 (10)	13.94	11.38–14.17 (8)	12.59
8th-11th day	12.75–17.31 (12)	14.87	13.65–14.87 (3)	14.06		
12th-15th day	11.16–16.81 (21)	14.33	12.90–17.31 (12)	14.77	11.96–14.32 (10)	13.29
16th-19th day	13.65–17.05 (11)	15.85	13.65–14.68 (4)	14.16		
20th-27th day	12.35–17.05 (17)	15.16	11.96–17.85 (14)	14.51	11.96–18.42 (10)	14.47

* The figures in parentheses give the number of observations. Other values represent grams of hemoglobin per 100 cc. blood.

A portion of the blood (2 cc.) was emptied into a centrifuge tube in which had been evaporated a solution containing 4 mgm. of neutral potassium oxalate and the remainder was emptied into a tared drying bottle, stoppered, and weighed at once on an analytical balance. All the blood determinations with the exception of water were made from the oxalated portion and were always completed a few hours after the blood was drawn.

The hemoglobin was estimated by the Newcomer method (16) using a Bausch and Lomb colorimeter. For the red blood cell count we used a Leitz counting chamber with a double Neubauer ruling. The blood pipette and the cover slip had been standardized by the U. S. Bureau of Standards.

[4] From E. R. Squibb & Sons.

We always counted 80 small squares and frequently double that number to check the accuracy of the count. All the counts were made by one operator.

For the determination of the red cell volume as well as for the measuring of the sedimentation index we used the method of Wintrobe (17.) This was essentially the Fahraeus method (18) except for the size of the tubes and the use of dry potassium oxalate as the anticoagulant. The Wintrobe method allowed us to determine red cell volume and refractive index on the same blood sample. The reading of the refractive index was done in a Pulfrich refractometer, new model. This instrument required about 0.30 cc. of plasma to make a reading. The water content of the blood was determined by drying a small sample, weighed into a light stoppered container, in an electric oven at 100°C. to constant weight. Reticulocyte smears were prepared and counted according to the new method outlined by Osgood (19) for oxalated blood. For differential counts smears on slides were stained with Wright's stain in the usual manner, 200 cells were counted.

The collected results of all of our data are presented graphically in chart 2. As it became obvious with the progress of our work that the anemia was most pronounced after the 16th day of pregnancy, most of our later analyses were limited to the period just previous to parturition. From the hemoglobin graph it will be noted that the anemia in general progressed in severity parallel with the development of the embryos. With the development of the anemia there occurred a fall in the red cell count and cell volume. Using a hemoglobin value of 14.83 per 100 grams of blood and a RBC count of 8,380,000 for the normals, we obtained an average color index of approximately 1, irrespective of the period of pregnancy. It was therefore evident that we were not dealing with an unbalanced production of hemoglobin and red cells.

Originally we had in mind to use the refractive index of blood plasma as an index of dilution because of the ease with which the refractive index can be determined, and the small amount of blood required. It, furthermore, had already been used by others (12, 20, 21) working with rats. Our data, however, show that while the hemoglobin, red cells, and cell volume values are all lower in non-pregnant animals, the refractive index increases markedly above that level for some days and then drops to a lower level a few days before parturition. By comparison with the graph of water content as determined by drying at 100°C. it appears probable that as a tendency to the production of hydremia develops, certain compensating mechanisms operate to maintain the blood solids constant. Ultimately, however, these prove insufficient and the refractive index falls with the hemoglobin and formed elements.

An intriguing change of values with advanced pregnancy was presented by the sedimentation index. The variability in the rate of sedimentation

of the formed elements of blood under different conditions was first called to the attention of medical practitioners by Fahraeus (18). He reported that while non-pregnant women gave a value of 3.3 mm. in one hour, pregnant women gave a value of 44.9 mm. The cause of this change was discussed by Fahraeus from the standpoint of changes in the specific gravity of corpuscles, the specific gravity of plasma, the size of corpuscles, the state of aggregation of corpuscles, the viscosity of the plasma, and the content of the latter in globulin. Since then a voluminous literature has accumulated on this subject without providing a satisfactory explanation.

Fahraeus did not mention any experiments with the rat. We have, however, found one reference (22) to experimental work in which five rats were used. The average sedimentation rate was reported as being very slow, namely, 6 mm. in 14 hours. The age, sex, and condition of the animals was not stated. It is evident from our work that the rat behaves like the human in the change in the sedimentation index with pregnancy.

While the literature on reticulocytes in humans is quite extensive, very little work has been done with rats. Scarborough (23) in his review in 1931 of the blood picture presented by normal laboratory animals makes the statement that the literature on reticulation in the rat yields no information on this subject. However, Beard, Baker, and Myers (24) in their studies on the nutritional anemia of the rat used the reticulocyte count as an important index. Employing the method of Friedlander and Wiedemer (25) they reported 3 per cent of the total red cells in young rats at weaning as being present as reticulocytes. When young rats developed an anemia due to nutritional deficiencies the reticulocytes increased. It was therefore thought probable that if the anemia in our pregnant females was not primarily a matter of dilution we might obtain a variation in their number.

From the results shown in chart 2 it appears that there may be a slight increase in reticulocytes during the latter part of pregnancy. We would be inclined to discount the increases observed if it were not for the fact that the values charted represent those obtained from a large number of animals. However, until we have obtained further data on the variability in reticulocyte count of normal animals under various conditions, we cannot be absolutely certain that the recorded response is due to pregnancy itself.

It appeared desirable to assemble some of our data in such a manner as to bring out the relations of one value to another more accurately. In chart 3 we have accordingly collected only those analyses which were taken from rats in which all the analyses were made on each rat. With these the picture is not altered in any essential feature. The refractive index still increased above normal only to fall below normal later, and the water content and sedimentation rate were markedly higher. The reticulocytes also do not indicate any definite nor sustained increase.

In table 2 we have calculated the data of chart 3 over into percentage deviation values, taking the values from non-pregnant animals as the base. This method of comparison shows a fair degree of parallelism between the hemoglobin, cell volume, and red cell values. However, while these values drop approximately 25 to 33 per cent with advanced pregnancy, the percentage of water increases only 4.8 per cent while the sedimentation index increases 580 per cent. This result, taken into consideration with the increase and decrease in the refractive index, shows that the anemia is far from representing a simple blood dilution.

We also made some post partum observations on the rats while they were nursing their young. The results of these observations are presented in chart 4. Shortly after parturition there occurred a loss in both hemo-

TABLE 2

Prepartum deviations of blood values from those obtained on non-pregnant animals

DAY OF GESTATION	HEMOGLOBIN	RED BLOOD CELLS	CELL VOLUME	REFRACTIVE INDEX	WATER	SEDIMENTATION INDEX	RETICULOCYTES
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
12th day (1)*	−7.12		−12.06	+1.54	+1.59	−2.42	
15th day (2)	−7.72		−14.48	+47.4	+2.08	+148.7	−15.0
16th day (6)	−9.14	−9.19	−9.74	+64.4	+1.07	+134.1	+2.0
18th day (4)	−8.60	−15.39	−13.50	+36.6	+1.48	+204.9	−25.0
19th day (13)	−18.34	−17.18	−24.24	+10.84	+2.77	+321.9	+100.0
20th day (15)	−26.61	−20.52	−24.49	−63.91	+3.50	+473.1	+90.0
21st and 22nd day (13)	−33.26	−25.17	−28.88	−10.61	+4.87	+580.4	+40.0

* Numerals in parentheses represent number of animals used for all values tabulated.

globin and cell volume, probably caused by hemorrhage, but the hemoglobin and cell volume returned to normal quite rapidly as the percentage of water decreased, which was probably effected primarily by lactation. The refractive index, however, showed a peak on the second day post partum, but all during lactation it failed to regain its prepartum level. The reticulocyte response was apparently positive, although due to the fact that our values were obtained almost two weeks after parturition, and with only four rats, we cannot come to any definite conclusions. A complicating factor in reticulocyte response is the variable occurrence of hemorrhage at parturition.

With an abnormal water content of the blood figuring so prominently in the low hemoglobin values in pregnant rats, we considered the possibility of the existence of a general state of hydration during pregnancy. We accordingly determined the water content of the carcasses of pregnant, non-

pregnant, and lactating rats,—in some cases after they had been deprived of drinking water for five days in addition. The determinations were made by killing the animals with ether, carefully removing the digestive and reproductive tracts without loss of fluid, fracturing the cranium and long bones, and then drying to constant weight at 90 to 100°C. in vacuo.

The results collected in table 3 reveal considerable differences in individuals with a decided increase in the water content of the pregnant animal. However, there may have been a tendency to greater conservation of water content in the pregnant rat when deprived of drinking water. It soon became evident that a complicating factor in the accurate determination of the water content was the rôle played by the fat stores. On

TABLE 3

The water and fat content of adult female rats

NOT DEPRIVED OF WATER									DEPRIVED OF WATER FOR 5 DAYS					
Non-Pregnant			Pregnant			Lactating*			Non-Pregnant			Pregnant		
No.	Water	Fat	No.	Water	Fat	No.	Water	Fat	No.	Water	Fat	No.	Water	Fat
	per cent			*per cent*			*per cent*			*per cent*			*per cent*	
3	64.0		6	60.0		147	67.7(72.2)	6.26	5	55.0		59	59.8(69.4)	13.9
4	64.0		7	61.0		148	68.6(73.1)	6.13	10	60.5(69.7)	13.3	37	62.6(68.4)	8.6
8	64.0(71.8)†	10.9	9	60.8		149	66.7(71.9)	7.28	139	58.3(71.9)	19.0	49	57.9(67.2)	13.9
141	57.3(71.5)	19.9	98	65.9(71.6)	8.0	150	61.3(72.7)	15.67	140	59.3(67.2)	11.8	116	62.4(67.8)	8.02
142	62.2(67.9)	8.5	51	60.3(73.1)	17.6									
			113	58.9(81.0)	27.6									
			81	60.8(73.5)	17.3									
Ave....	62.3(70.4)	13.1		61.1(74.8)	17.6		66.0(72.5)	8.83		58.2(69.6)	14.7		60.6(68.2)	11.1

Determinations were made on the total rat minus the digestive and reproductive tracts. The pregnant females were killed on the 21st day of gestation.

* The lactating rats were killed when the young were 12 to 13 days old. Three cubic centimeters of blood had been removed from each before killing,—this was not sufficient to affect the determinations of water critically.

† The numerals in parentheses represent the percentage of water calculated on the fat-free basis.

the whole the pregnant rats contained more fat. We accordingly extracted the dry carcasses of our animals with ether in Soxhlet extractors for a week so that we could recalculate our data on the fat free basis. When this correction was used it appeared that the pregnant rats had greater reserves of water than the others (26, 27). Lactating rats also contained more water. When deprived of water, both pregnant and non-pregnant rats gave about the same values. However our data are too limited to allow final conclusions.

The failure to get pronounced differences in water content of the entire animal led us to run some determinations on various tissues such as spleen, liver, kidney, muscle, and skin, with the expectation that certain tissues

might reveal much greater differences than others. Our data on this are also limited (table 4) but they suggest an increase in the water content of the skin, especially that taken from the abdominal region, care being taken not to include any mammae. Muscle tissue gave similar results. We do not feel justified to draw conclusions from analyses of spleen, liver and kidneys, because of variations in blood content and the well known contractility of the spleen, although with the liver and kidney we clamped the blood vessels previous to excision. Drenching a male rat with 7 cc. of water by mouth and then removing the tissues 30 minutes later also gave no pronounced deviation from normal values, although the values obtained

TABLE 4

Water content of blood and other tissues of rats as influenced by pregnancy and other conditions

	RAT NUMBER	SPLEEN	LIVER	KIDNEYS	MUSCLE		SKIN		BLOOD	HEMOGLOBIN	SEDIMENTATION INDEX	REFRACTIVE INDEX
					Psoas	Leg	Dorsal	Ventral				
		per cent H_2O	*per cent* H_2O	*per cent* H_2O	*per cent* H_2O	*per cent* H_2O	*per cent* H_2O	*per cent* H_2O	*per cent* H_2O	*grams*	*mm.*	
Non-pregnant female	136	78.0	74.35	74.6	75.7	70.77	55.28	40.76	78.98*	14.83*	3.6*	1.35151*
Pregnant female	84	78.88	72.47	76.74	77.01	72.74	56.39	49.35	82.97*	10.70*	25.1*	1.35030*
Pregnant female	104	79.98	74.25	77.88	76.8	73.36	57.83	55.3				
Male	169		73.28	76.32	75.96	71.5	61.8	38.6				
Male	172	77.6	71.46	75.16	76.34	72.2	59.6	49.2		15.25		
Male	173	78.46	72.07	77.9	76.8	65.6	61.8	61.5		15.06		
Male deprived H_2O 24 hrs	179	80.56	70.90	76.06	75.49	71.31	59.06	56.92	78.5			1.35497
Male flushed with 7 cc. H_2O	174	78.83	71.86	76.52	77.15	73.52	53.32	78.36	79.53	15.67	4.0	

* These values represent total averages of all the females. The pregnant females were killed on the 21st day of gestation.

for blood and muscle were somewhat higher than those obtained from a rat which had been deprived of water for 24 hours.

SUMMARY

An anemia of pregnancy occurs in rats which becomes most pronounced after the 16th day of pregnancy and reaches the greatest severity at parturition. Undoubtedly the anemia at parturition is accentuated by hemorrhage incidental to the birth of young.

After parturition there is a rapid recovery from the anemia.

The anemia of pregnancy appears not to be of dietary origin, since it was found impossible to correct it by additions of copper, iron, manganese, iodine, arsenic, yeast, dried beef liver, fresh egg yolk, and cod liver oil to a ration satisfactory for reproduction.

The refractive index of the blood plasma increased markedly for some days during pregnancy and dropped to a level below normal a few days before parturition.

The sedimentation index of the formed elements of the blood increased markedly with the advance of pregnancy.

The percentage of water in the blood increased about 4 per cent during the later stages of pregnancy. This did not represent a simple hydration phenomenon because according to the refractive index certain compensating factors increased the blood solids as the tendency to hydration developed.

The reticulocyte count was not increased markedly, if at all, therefore it cannot be definitely concluded that there occurred stimulation of hematopoiesis with advanced pregnancy.

There occurred no unbalanced production of red cells or hemoglobin during pregnancy inasmuch as the color index remained constant at approximately 1.

The water content of the carcasses of pregnant rats calculated on a fat free basis was higher than in non-pregnant rats. It was also higher in lactating rats. When deprived of water, pregnant and non-pregnant rats gave about the same value. Although our data are limited in number, it appeared that the water content of the skin and muscle tissue accounts for the difference.

It is suggested that in an analysis of an anemic condition in the human during pregnancy it might be advisable to give consideration to the picture being complicated by an hydremia.

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ADAPTATION OF SEXUAL ACTIVITY TO ENVIRONMENTAL STIMULATION[1]

CORDELIA OGLE

From the Laboratory for Experimental Medicine, University of Cincinnati

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The influence of climate on the human glands of internal secretion has been previously reported (1). The sex glands are affected along with the others, the onset of the menses occurring at an earlier age in the more stimulating regions, with fertility reaching an early peak and then declining more rapidly than in the less stimulating zones. Due to the probable inaccuracies encountered in human statistics, the present work was undertaken in order to determine whether similar results would be obtained with laboratory animals under controlled conditions, where any check on the functions of the sex glands by artificial procedures would be discarded. We now have experimental data on white mice verifying most of the human findings.

EXPERIMENTAL CONDITIONS. Two constant temperature rooms, identical in construction, were erected and have been described elsewhere (2). One was kept at 60–68°F. with a very short temperature cycle so that the mean temperature of 64°F. predominated most of the time. The other was kept at 88–92°F. with the humidity near 75 per cent. The control room had a temperature range of 70–80°F. No record was made of the humidity in the cold or control rooms.

A single strain of white mice was bred in the control room and at twenty-one days of age the young were separated from the mothers and placed in the various groups for observation. Such mice, allocated to the various groups, are, in this paper, termed "immigrants" to the respective groups. The offspring of the "immigrants" to a group are, in this work, referred to as the "descendants" of the given group. There have been five experimental groups, namely: control room group, hot room group, cold room group, and two change groups. One change group (change group I) spent twelve

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consecutive hours a day in the hot room and the remaining twelve in the cold room; the second change group (change group II), like the first, spent twelve consecutive hours in the hot room, but for the remainder of the day was shifted back and forth between the hot and cold rooms three times, spending a total of sixteen hours in the hot room and eight hours in the cold room each day. The female mice were tested as to fertility with control males, while, for the male mice, interest was chiefly directed toward the rate of growth and data on this latter point are presented in another article. After suckling the young for twenty-one days, the mothers were rested seven days before they were remated. There have been indications that the fertility of the males in this experiment has been affected, as reference to a later paragraph will show.

The cages were of $\frac{1}{4}$ inch mesh galvanized wire, 9x9x15 inches with a closed food bin. Keller (3), in his book, *The Laboratory Mouse*, describes a closed food bin for mouse cages, but the type used here was described by him in a private communication. It consists of galvanized wire of $\frac{1}{3}$ inch mesh forming a closed sloping trough near the top of the cage in the rear with food admitted to the bin through a small opening in the top of the cage. Maternity cages suitable for mice were constructed as a definite and convenient means of determining litter size. These cages were made in the same manner as the breeding cages except for the bottoms, which were of $\frac{1}{2}$ inch mesh wire. Trays, 2 inches deep, the same in length and width as the maternity cage proper and lined with paper toweling, served as the true floor or bottom for these special cages, since the young would fall through the large meshes of the false floor. Pregnant females were placed in the maternity cages on about the second or third day prior to parturition as calculated by the date of the vaginal plug. The mother and young were moved from the maternity cage to the regular cage soon after the delivery. Paper toweling was used as the nesting material.

Purina dog chow and water were given freely, and lettuce on alternate days to all animals. The mothers received, in addition, a few sunflower seeds daily and small amounts of lean raw beef once a week. The animals were weighed each seventh day after the day of separation from the mothers. Weighings were carried out to the nearest tenth of a gram. The females were mated with control males very soon after the rupture of the vaginal plate. The first oestrus and the length of the oestrous cycle were determined by the usual vaginal smear method.

Experimental results. Dealing first with the time of onset of sexual functions, it is seen from the data in table 1 that the vaginal plate ruptured at practically the same age for the cold room group and control room group females, whereas this phenomenon for the hot room group "immigrants" and the cold room group "descendants" occurred at a later age. The difference is slight, however, and not mathematically significant. The

change group females occupied an intermediate position. It was difficult to get the hot room group females to care for their young, hence the small number of hot room descendants for which only the arithmetical values of the age of plate rupture and first oestrus are given. Maturity with them was much delayed.

The first litters delivered by the females of the control and cold room groups were of practically the same size. The control group females, during their first five litters, brought forth about an equal number of young each time, but the cold room group females increased their litter size beyond the original figure and, at the age of 225 to 364 days, delivered litters averaging about eight young. The cold room descendant females were

TABLE 1

Mean ages of females at rupture of vaginal membrane and first oestrus

GROUP	NUMBER OF ANIMALS	AGE AT PLATE RUPTURE	AGE AT FIRST OESTRUS
		days	*days*
Control	40	32.04 ± 2.035	33.22 ± 0.976 (18)
Hot room:			
"Immigrant"	59	34.74 ± 1.0397	35.76 ± 1.129
"Descendant"	9	41.4*	46.16*
Cold room:			
"Immigrant"	25	32.82 ± 1.500	33.42 ± 1.553
"Descendant"	41	34.1 ± 1.312	35.12 ± 1.116
Change	37	33.52 ± 1.125	35.28 ± 1.335

* Arithmetical averages.

The age at first oestrus was determined for eighteen of the forty control females.

fertile at an earlier age than the females of any group. The females of the hot room and change groups had the lowest fertility, as shown by table 2, and gave birth to many dead young.

Four hot room group males were mated with hot room females (thirty-five to fifty days of age). Each male was mated with from five to seven females. There were eighteen successful pregnancies, with an average litter size of 3.33 young (a total of sixty young in the eighteen litters). Twenty hot room group females (thirty-five to fifty days of age) were successfully mated with control males in the hot room with 112 young born, or an average litter size of 5.6 young. Thus smaller litters were born when the hot room group animals were inbred than when they were mated with control room animals.

After the delivery of their first litters, females of the hot room and change

groups, although repeatedly mated, bore scarcely any young. In the case of the former, it seemed that the low sexual functionings were engendered by the constant depressive conditions in the hot room and, therefore, perhaps a sojourn in the cold room would be beneficial. For this test, eight hot room females, at an average age of 167 days, were placed in the cold room and mated with control males. Within thirty days, these females had borne litters averaging 7.75 young each, or an increase in fertility of

TABLE 2

Average litter size

LITTER SEQUENCE	20 CONTROL ROOM FEMALES	45 HOT ROOM FEMALES	21 "IMMIGRANT" 23 "DESCENDANT" COLD ROOM FEMALES	21 CHANGE I GROUP FEMALES	21 CHANGE II GROUP FEMALES
1	6.8 young‡ (20)* [84.2 da]	‡5.6 young (20) †3.33 young (18) [98.5 da]**	6.71 young‡ (21 "I") [94.6 da] 6.91 young‡ (23 "D") [78.7 da]	2.3 young‡ (3) [94 da]	5.25 young‡ (8) [93.2 da]
2	6.3 young‡ (18) [132.27 da]	5.1 young‡ (9) [145 + da]	8.43 young‡ (14 "I") [138.1 da]	*Changed to Cold Room* 4.11 young‡ (9) [158 da]	2.66 young‡ (3) [187 da]
3	7.1 young‡ (15) [177.4 da]	*Changed to Cold Room* 7.75 young‡ (8) [197 + da]	8.0 young‡ (7 "I") [211.6 da]	*Changed to Hot Room* 6.0 young‡ (5) [179 da]	*Changed to Cold Room* 4.3 young‡ (3) [308 da]
4	6.5 young‡ (13) [234.4 da]		*Changed to Hot Room* 4.8 young‡,† (6 "I") [178 da]		*Changed to Hot Room* 6.9 young‡ (10) [187 da]
5	6.4 young‡ (8) [265.13 da]				
Number fertile at 100 days of age..	20 (100%)	38 (84.4%)	21 "Immigrants" 23 Descendants (100%)	3 (14%)	8 (38%)

* Figures in parentheses indicate the number of cases.

** Figures in brackets indicate the average age of mothers at delivery of young.

‡ Females were mated with control males.

† Females were mated with hot room group males.

97 per cent for the inbred hot room females and an increase of 34 per cent for the hot room females mated with control males in the hot room. As table 2 also shows, six cold room females were placed in the hot room at an average age of 152 days. Three were mated with control males and three with hot room males. At an average age of 178 days, these females had borne young, the number in each litter being 4, 2, 7, for the former matings and 5, 5, 6 for the latter. Hence, the average litter size was 4.8 young, whereas the previous average number of young delivered by these females in the cold room was 8.3. Thus by mating the cold room females in the

hot room, with either hot room or control room group males, the result was a decrease of 41 per cent in litter size.

Altogether there were forty-two change group females and only eleven of them had borne young at ninety-four days of age, this low fertility indicating functional exhaustion of the sex glands. The sudden daily change from a hot, moist environment to a steady cool state was far too severe for the females of the change I group as attested by the fact that only three out of 21 (14 per cent) of them were able to reproduce under these strenuous conditions. In order to determine whether these females, at one hundred and twenty-one days of age, had been rendered sterile by the environmental conditions, the shifting was discontinued and they were kept in the cold room for a period of four to six weeks. Nine (42.8 per cent) of these females bore young with an average litter size of 4.11 young. Thus, subjection to the steady cool environment immediately after the period spent under the shifting conditions enabled more females to be successfully mated and to bring forth a greater number of living young in each litter. But as yet only twelve of these change I group females had borne young. The remaining nine that had failed to reproduce by an average age of one hundred and forty-three days were mated in the hot room with control males. Five of these bore young with an average litter size of six young, which was the largest litter size for change I females under any conditions.

The change II group females were rested in the hot room for a greater length of time each day than the change I group animals and, hence, were more fertile than the latter while undergoing the respective shifting routines. For this reason, when the shifting of the change II group females was discontinued and they were kept in the cold room, only three of them bore young and with an average litter size of 4.3 young; this was a decrease in litter size of near twenty per cent. Ten of these females were mated in the hot room with control room group males and bore litters averaging 6.9 young each. The steady moist heat of the hot room was quite a tonic to the reproductive processes of these over-stimulated females since both change groups were more fertile during their sojourn in the hot room than under any other environmental conditions to which they were subjected.

There was noted a most interesting behavior of the male mice in regard to the use of their scrotal sacs. It is commonly considered that retention of the testes within the body cavity leads to sterility, but here the testes of the cold room group males remained in the abdominal cavity so long as the mice were kept in the cold room. Those of the hot room and change group males rested in scrotal sacs which depended far. However, the cold room group males which were mated in the hot room developed far depending scrotal sacs within three days after their admission to the hot room. The testes of the control room group males rested in scrotal sacs which did not depend as far as those of either the hot room group or change group males.

Although retaining their testes within the abdominal cavity, the males in the cold room were fully as fertile as the controls, and more fertile than those in the hot room with well descended testes.

Discussion. Thus we have data on laboratory animals which bear out the observations on the human being to the effect that climatic stimulation markedly influences the activity of the sex glands. The steady coolness of the cold room produced an increase in the fertility of the females there, whereas the constant heat and high humidity of the hot room were conducive to small litters with most of the young born dead. The fertility of the cold room females was markedly decreased by mating them in the hot room and keeping them there during the gestation period, while a sojourn of equal duration in the cold room brought about a pronounced increase in litter size for hot room group females.

The shifting environmental conditions tested during this experiment were most unfavorable to reproduction. However, either constant moist heat or a steady cool environment enabled these change group animals, in most cases, to increase their fertility, the former condition being decidedly more beneficial than the latter. In time, the conditions prevailing in the hot room would very probably bring about a reduction in the newly acquired fertility of these females (originally of the change groups) down to that of the regular hot room group females. There seemed to be a definite reproductive exhaustion in these mice submitted to the most changeable and excessive stimulation.

Sundstroem (4) kept male mice under humid and stagnant air conditions for six months. At the end of this period, the testes presented a state indicative of sterility. His hot room males had large scrotal sacs which he considered as accessory cooling organs. Moore (5) found that the scrotal temperature was 1 to 1.5°C. lower than the abdominal temperature for normal rats, guinea pigs, and rabbits. Forced retention of the testes in the peritoneal cavity gave rise to progressive degeneration of the entire generative portion of the guinea pig within five to seven days, but, a few months after the return of the testes to the scrotum, regeneration occurred. He concluded that the function of the scrotum was that of a heat regulator for the production of sperm. The depressive temperature and humidity of the hot room, in the experiment here reported, permitted only a very low fertility even though the males had very large scrotal sacs. On the other hand, the testes of the cold room males remained in the abdominal cavity, and these animals were equally as fertile as the control room group males. The testes of the males of all the other groups rested in scrotal sacs, which for the hot room males depended most. It would seem that retention of the testes intra-abdominally is not necessarily harmful, unless over-heating occurs.

It is now considered that there are gonadal, hypophyseal, and adrenal

interrelationships, but the fine points of these interactions are by no means clear. The sexual functionings under the various conditions here presented are believed to be dependent on changes in these interrelationships, indirectly affecting the sex glands, with the adrenals probably initiating the responses to changes in environmental stimulation.

SUMMARY

1. White mice subjected to a warm humid environment show a low fertility in three ways, namely: a low percentage of matings that result in pregnancy, small litter size, and low viability of offspring.

2. Most efficient sex functioning comes with a steady cool environment. There the greatest number of matings result in conception, large litters of lusty offsprings are born, and the onset of sexual life and fertility in the young come earlier than in the moist heat.

3. Mice seem poorly adapted to a changeable environment, and suffer a severe loss of sexual function when thus over stimulated. This loss is not permanent, however, but is replaced by normal fertility when they are relieved of the exhausting stimulation.

4. Adaptation to different temperatures is quickly accomplished by mice, for within a week after shifting animals from one environment to another they assume the fertility level characteristic of their new "climate."

5. In the cold room, males keep their testes within the abdomen, while in the moist heat they are far dependent in loose scrotal sacs. With fertility highest in the cold room mice, it is evident that failure of the testes to descend from the abdomen does not in itself produce sterility. This difference between the hot and cold room males is more likely a matter of adaptation to keep the testes nearest the optimal temperature for proper function.

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CLIMATIC INFLUENCE ON THE GROWTH OF THE MALE ALBINO MOUSE[1]

CORDELIA OGLE

From the Laboratory for Experimental Medicine, University of Cincinnati

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A relationship between climatic stimulation and the activity of the human glands of internal secretion, based on statistical data, has been reported by Mills (1). In the human being, the onset of the menses occurs at an earlier age in the more stimulating regions, with fertility reaching an early peak and then declining more rapidly than in the less stimulating zones. An investigation of climatic influence on the sex functions of the albino mouse was undertaken in an attempt to determine the accuracy of human statistics, the result of this experiment agreeing closely with the human findings. Coincidentally with these observations on the sex functions, it was also noted that body weight and length were greatly influenced by environmental stimulation. Conditions of moist heat, by affecting the general metabolic level, were found to produce definite alterations in the rate and type of growth, changing the ratio of body length to body weight and the length relations of body and tail. The findings would seem to be of basic importance in animal husbandry as well as in problems of human growth under different climatic conditions.

EXPERIMENTAL CONDITIONS AND RESULTS. The conditions of the experiment are described in the preceding article which dealt primarily with the sex changes produced by the different artificial "climates." In the following paragraphs attention is directed to differences in growth and development. Body and tail length measurements were made only on freshly killed males.

There were observed marked differences in the growth rates of the five groups of mice. The weight curves for the males of the control room and cold room groups fall very close together, with the cold room "immigrants"

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remaining slightly but consistently below the other two groups. The hot room males made their greatest increase in body weight during their first week of residence in the hot room. Thereafter, they gained but slowly, soon reaching a plateau and, by eighteen weeks of age, had begun to decline. The growth curve of the change II group males closely followed that of the hot room males until the point where the latter reached their plateau, from

TABLE 1

Weekly weights in grams of males

AGE	CONTROL	HOT ROOM GROUP	COLD ROOM GROUP		CHANGE I GROUP	CHANGE II GROUP
			"Immigrants"	Descendants		
weeks						
3	8.16±0.409*(50)	8.64±0.312(48)	8.35±0.492(24)	8.31±0.347(40)	7.71±0.410(24)	8.43±0.607(22)
4	12.44±0.434 (50)	12.46±0.430(48)	11.10±0.568(24)	10.60±0.382(40)	9.87±0.477(24)	12.0 ±0.773(24)
5	17.1 ±0.368 (50)	14.41±0.438(48)	14.67±0.940(24)	15.56±0.573(40)	12.12±0.589(24)	14.44±0.198(29)
6	19.56±0.453 (50)	15.78±0.154(48)	18.6 ±0.873(24)	19.20±0.554(39)	13.69±0.615(24)	15.56±0.540(27)
7	21.06±0.529 (50)	16.45±0.488(45)	19.37±0.774(24)	21.50±0.591(39)	14.91±0.640(24)	16.92±0.534(28)
8	22.22±0.633 (50)	16.88±0.480(45)	21.39±0.484(24)	21.93±0.561(40)	16.00±0.623(24)	17.06±0.817(23)
9	23.08±0.546 (50)	17.89±0.714(33)	22.7 ±0.666(24)	22.76±0.768(27)	16.10±0.676(24)	17.41±0.879(23)
10	23.68±0.474 (50)	19.26±0.545(32)	23.6 ±0.663(24)	24.54±0.963(27)	17.41±0.662(24)	18.41±0.926(22)
11	24.32±0.518 (50)	19.88±0.679(31)	24.2 ±0.761(24)	24.62±0.707(27)	18.02±0.532(24)	19.13±0.539(22)
12	25.27±0.537 (43)	19.75±0.682(32)	25.1 ±0.757(24)	25.60±0.688(27)	18.87±0.568(24)	20.43±0.539(22)
13	25.64±0.689 (43)	20.16±0.765(22)	25.6 ±0.728(20)	25.39±0.658(18)	19.58±0.523(23)	21.45±0.723(22)
14	26.12±0.734 (42)	20.59±0.483(22)	25.9 ±0.765(20)	26.41±0.784(18)	19.81±0.614(23)	21.86±0.511(22)
15	26.98±0.533 (42)	20.91±0.596(22)	26.0 ±1.067(20)	26.58±0.645(18)	19.92±0.559(23)	22.47±0.586(22)
16	27.19±0.745 (42)	20.51±0.720(19)	26.9 ±1.027(20)	26.83±0.713(18)	20.42±0.639(23)	21.54±0.455(22)
17	27.28±0.458 (42)	21.17±0.946(13)	26.39±0.674(17)		21.16±0.596(18)	22.02±0.629(18)
18	27.47±0.569 (42)	20.58±0.914(12)	26.87±0.690(16)		21.47±0.662(18)	22.83±0.577(18)
19	27.29±0.657 (42)	21.04±0.874(12)	27.37±0.678(16)		21.66±0.545(18)	22.80±0.697(18)
20	27.81±0.708 (42)	20.79±0.820(12)	27.62±0.718(16)		22.12±0.682(16)	23.97±0.664(17)
21	28.14±0.539 (42)				21.46±0.642(12)	
22	27.5 ±0.622 (38)				22.29±0.863(12)	
23	27.95±0.649 (38)				22.25±0.976(12)	
24	27.58±0.612 (38)				22.7 ±0.837(12)	
25	29.09±0.617 (27)					
26	28.58±0.747 (26)					
27	28.54±0.772 (24)					
28	28.04±0.828 (24)					
29	28.08±1.029 (24)					
30	29.25±0.739 (24)					
31	29.13±0.801 (23)					
32	29.36±0.566 (22)					

* Figures in parentheses indicate the number of cases.
The steady diminution in group numbers was due to killing off for length measurements.

this point on the change II group males surpassed the hot room males. The change I group males grew at a slower rate than any of the other males, but their growth was steady, so that, near eighteen weeks of age, they surpassed the hot room males. The mean weekly weights for the various groups are presented in table 1, and chart 1 was constructed from these data.

Concomitant with these different rates of increase in body weight there

were found significant differences in the growth of the body skeleton of the mice from the various groups. Table 2 shows that all males, except those of the change groups, had reached their mature body form by twelve weeks of age. The hot room group males exhibited the shortest bodies and

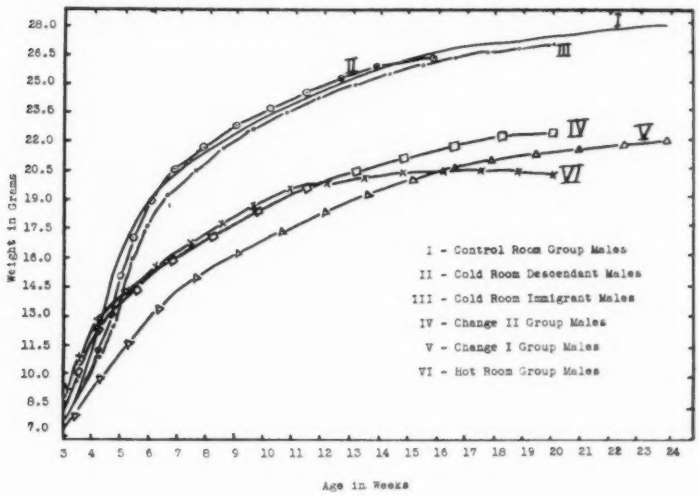

Chart 1

TABLE 2

Body and tail lengths of males according to age and groups

AGE	CONTROL GROUP		HOT ROOM GROUP		COLD ROOM GROUP		CHANGE GROUP	
	Body L.	Tail L.	Body L.	Tail L.	Body L.	Tail L.	Body L.	Tail L.
weeks	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
8			8 2 (13)	9 3	8 5 (13)	7 7		
12	8 45 (11)	9 1	8 7 (14)	9 8	9 1 (13)	8 2		
16			8 4 (11)	9 4	9 1 (9)	8 2	8 6 (8)	9 3
20			8 5 (11)	9 2	9 0 (13)	8 0	8 7 (10)	9 1
24	9 3 (10)	9 1					8 9 (9)	9 5
28							8.98 (9)	9 5
32							9 0 (11)	9 6

* Figures in parentheses indicate the number of cases.

longest tails of any group; the tails were always 1 cm. longer than their bodies whereas the converse applied to the cold room males. Plate 1 offers a nice illustration of this point. From the twelfth to the twenty-fourth week of age, the bodies of the control room males had increased in length

approximately 1 cm. but the tail length remained unaltered during this period. The body and tail lengths of the change group males, during their early growth, approximated those of the hot room males, but the

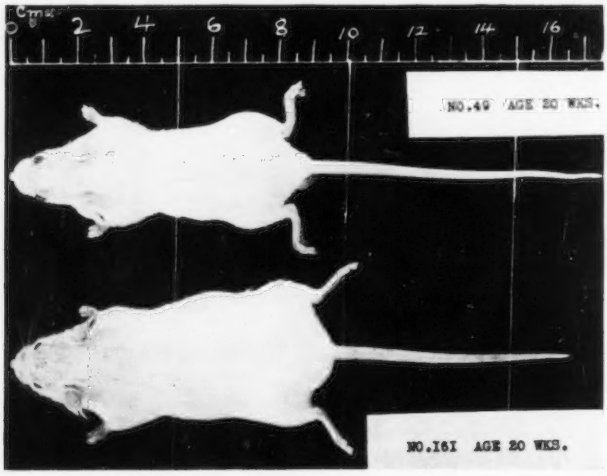

Plate 1. Comparison of representative hot and cold room males at 20 weeks of age. Males 49 and 161 were born in the control room. At 3 weeks of age no. 49 was placed in the hot room to remain there until 20 weeks of age, and no. 161 was placed in the cold room for an equal-period of time.

TABLE 3

Male body weight : Body length ratio

AGE	CONTROL GROUP	HOT ROOM GROUP	COLD ROOM GROUP	CHANGE GROUPS
weeks	*cm./gm.*	*cm./gm.*	*cm./gm.*	*cm./gm.*
8		0.453 (12)*	0.381 (13)	
12	0.418 (11)	0.426 (14)	0.341 (13)	
16		0.480 (11)	0.336 (9)	0.427 (8)
20		0.432 (11)	0.324 (13)	0.380 (10)
24	0.349 (10)			0.370 (9)
28				0.427 (9)
32				0.381 (11)

* Figures in parentheses indicate the number of cases.

former continued to grow steadily and, at thirty-two weeks of age, both their body and tail lengths were near the maximum recorded during this experiment for animals of any group.

Arithmetical values for the relationship between the body weight and body length of these males are expressed in table 3. Ordinarily, as an ani-

mal ages, the value of this ratio declines due to the continued deposition of adipose tissue long after the bony skeleton has stopped growing. Thus, we see that the ratio for the control room males, at twenty-four weeks of age, is numerically less than when they were twelve weeks of age. Due to the fact that the hot room males had exceedingly light and short bodies their ratio had the highest numerical value and varied little from one age to another. There was a steady but slow decline in the weight: body length ratio for the cold room males, since they, like the control males, had heavy bodies. The change group males possessed relatively light but long bodies, hence this relationship is expressed by a fairly high figure which gradually decreased as the animals grew older.

Discussion. These differences in growth are probably due to changes in the thyroid-pituitary activities, which are, in turn instigated by the environmental temperature and humidity. The steady coolness of the cold room and the environmental conditions of the control room stimulated the occupants of these rooms to make increments in weight and body length at a more rapid rate than did any other set of environmental conditions here reported.

According to Sundstroem (2), the tails and scrotal sacs serve as heat radiating organs. The tails of the cold room mice were very short, but proximally were larger in diameter than those of any other animals of this experiment; this phenomenon appears as an adaptive measure in order to check the loss of body heat by radiation. Conversely, perhaps as an adaptive device, the hot room animals had very long spindle-like tails so as to increase their surface for cooling. Likewise, the slight bodies of the hot room mice would seem to be an adaptation in order that they might avoid overheating. The males in Sundstroem's experiment had scrotal sacs which depended far, as did also those of the hot room mice of this experiment. However, the testes of the cold room males remained in the abdominal cavity so long as the mice were in the cold room, so that the scrota were only slightly developed, but a sojourn of three days in the hot room was ample time for the production of large scrotal sacs. The change group males ranked near the hot room males as to scrotal size, with those of the control room males being intermediate between those of the latter group and the cold room males.

Sumner (3) studied some effects of heat and cold upon white mice. The temperature of his warm room varied from 75–80°F., and the cold room from 43–48°F. At the temperature of his cold room, he did not find any definite effect upon body length, probably because it was quite below the optimal temperature, but he did discover that the cold room mice had distinctly shorter tails than the warm room mice. His data on mouse growth agrees with that of table 2 of this paper, both of which indicate that comparisons of earlier and later measurements upon the same animals

show a distinct tendency toward the reduction of these experimentally produced differences during subsequent growth, even when the conditions remain unchanged. This statement applies only to the cold room and hot room group males, since the change group males were still showing increases in body and tail lengths at thirty-two weeks of age. The change II group males responded better in body weight than did the change I group as the regime imposed upon the former was less strenuous than that for the latter group. However, the environmental conditions for both groups were so severe that growth was delayed, but, at a comparatively late age, these animals possessed body and tail lengths near the maximal values in this experiment.

SUMMARY

1. Mice adapted to a steady cool environment increased in body weight and body length at practically the same rate as control mice. However, these cold room mice had tails fully 1 cm. shorter than those of the control room males.

2. Mice subjected to continuous moist heat had exceptionally short slender bodies, the tails of each being 1 cm. longer than the body.

3. A changeable environment retarded the growth of male mice, but, at a late age, such animals attained both great body and tail lengths.

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AN EXPERIMENTAL STUDY OF THE MOVEMENTS OF THE SMALL INTESTINE[1]

KENNETH B. CASTLETON[2]

From the Division of Experimental Medicine, The Mayo Clinic, Rochester, Minnesota

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METHOD. Dogs were used which had been prepared according to the method described by Biebl. A loop of intestine from the desired site was brought out on the abdominal wall, its blood and nerve supply, as well as its continuity with the rest of the intestinal tract, intact. The loop was surrounded by a bipedicled skin flap, and the abdominal wall was closed beneath it. The loop, of such length that about 15 cm. was available for observation, was taken from one of four sites 30 and 100 cm. distal to the ligament of Treitz, 70 and 30 cm. proximal to the cecum. The movements in this exposed intestinal loop were observed directly, and kymographic and electro-enterographic records were made. The kymographic records were obtained with the usual receiving and recording tambours; the receiving tambour was clamped to the loop. The surgical procedure was varied slightly in order to obtain the electro-enterograms. Instead of surrounding the intestinal loop with skin it was placed under the skin of the abdominal wall, and at a later date a piece of the overlying skin was excised to expose a portion of the loop. The recording apparatus consisted of a pair of silver wire electrodes coated with silver chloride to make them nonpolarizable, connected with an ordinary string galvanometer of the Hindle type.

The animals were carefully trained before making observations. They would lie quietly and comfortably on their sides on a padded bed, without exhibiting the least disturbance when observations were being made.

The diets used were as follows: The protein meal consisted of lean horse meat. The carbohydrate meal was composed of a mixture of milk and syrup of about equal portions. The fat meal was either cream and egg yolks, or equal parts of lard and cracker meal. The amount given varied with the size of the animal. The animal usually was on the specific diet for two days before observations were made. Before each experiment the

[1] Abridgment of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Fellow in Surgery, The Mayo Foundation.

animal was fasted, usually sixteen hours, and the rate of intestinal movements was estimated in the fasting state. The specific meal was then given and the intestinal movements were observed throughout the digestive period. In some instances the animals were fasted for as long as forty-two hours, but the results did not differ from those following the shorter fast. The cathartic used was castor oil in doses of 30 cc.

RESULTS. *Direct observation.* It was apparent, on direct observation, that the most common movement of the small intestine is rhythmic, of which several forms exist. One form is rhythmic segmentation, which at times is clear cut, and at other times is less distinct; it rarly occurs in the dog with such regularity as that observed by Cannon in the cat. Another form is a to-and-fro movement, consisting of a short wave which sweeps at first forward and then backward. The loop may be the seat of several of these movements at the same time. These two types of movements are seen most commonly in the distal part of the small intestine, and their function is obviously that of mixing rather than of progression. A third type of movement is distinctly progressive, also rhythmic, but consists of a series of waves, each passing a short distance, each succeeding one beginning just caudal to its predecessor. Six or ten of these waves may be involved before the entire loop is traversed. Before the distal end of the loop is reached by one series, the proximal end may become involved in another series which proceeds in exactly the same manner. Or the entire loop may remain quiet for a few seconds before another series of waves appears at the proximal end. In other cases, each of these rhythmic waves may sweep through the entire loop. These progressive waves are seen much more commonly in the proximal portion of the intestine.

Kymographic records and electro-enterograms. The rates, as recorded kymographically and electro-enterographically, were practically the same, and the data secured by these two methods, for the most part, can be considered together.

Frequency of rhythmic movements. The frequency of rhythmic movements was very distinct. A definite gradient was found in the dog, as Alvarez had noted in the rabbit. The rate for a loop 30 cm. distal to the ligament of Treitz was remarkably uniform at 17 to 18 each minute. The rate of a loop 100 cm. distal to the ligament of Treitz was 15 to 16, while that of a loop 30 cm. proximal to the cecum was 12 to 14 each minute (table 1).

The effect of ingestion of food on intestinal movements. In order to determine the effect of taking food on intestinal rhythm, a series of records was made with the animal in the fasting state, then a continuous record was made when the animal was fed on the table. Twenty-three records were made. In nine of these a prompt increase in the movements occurred before the animal had finished the meal, which rarely took two minutes. In

nine other records there was a well-marked increase within twenty minutes. This prompt increase occurred regardless of the site of the loop being used. In fact, in three experiments on an animal with the loop 70 cm. proximal to

TABLE 1

*Rates (per minute) of rhythmic contraction of different portions of small intestine of the intact dog**

SITE OF LOOP	KYMOGRAPHIC RECORDS					ELECTRO-ENTEROGRAPHY RECORDS				
	Fasting	Meat	Carbohydrates	Fat	Cathartic	Fasting	Meat	Carbohydrates	Fat	Cathartic
30 cm. distal to ligament of Treitz	16–18	17–18	16–18	17	16–18	17–19	17–18	17–19	17–19	17–18
100 cm. distal to ligament of Treitz	14–17	15–16	14–17	14–15	15	15–16	15–16	15–17	15–17	15
70 cm. proximal to the cecum	14–15	12–15	12–15	13–14	12–15					
30 cm. proximal to the cecum	12–13	12–13	12–13	12–13	12	12–13	12–13	13–14	12–13	13–14

* During fasting, after representative diets, and after cathartic, as determined from kymographic and electro-enterographic records. Uniformity of the rate, regardless of the digestive state, and also the gradient of rhythmicity are noteworthy.

Fig. 1. Kymographic record of movements of loop of intestine 30 cm. proximal to the cecum. The animal was fasting, and movements of the intestine were hardly discernible. At a signal the animal was fed meat, and immediate increase in amplitude of movements was noted.

the cecum, there was a good increase in the movements within three minutes in each experiment (fig. 1).

Intestinal movements after various diets. No distinctive difference was noted in the rate of the rhythmic movements of the intestine with different diets. The amplitude of contractions, and the total activity of the loop,

varied greatly with different dogs on the same diet and in the same dog on the same diet on different days. There was, however, a marked difference between the fasting animal and one which had recently eaten, regardless of the site of the loop. In the fasting animal the loop was usually quiet most of the time, with movements hardly discernible. These quiet periods were interrupted at irregular intervals by periods of activity, usually lasting only a few minutes or even a few seconds. In other words, there is a definite tendency for the movements to occur in cycles, periods of activity alternating with periods of rest, the rest periods predominating. I have seen a loop remain quiet except for a very occasional, faint, localized contraction, for an hour and forty-five minutes. This is in marked contrast to the activity of the loop in an animal which has eaten recently. In the latter case the loop is active most of the time, the movements are stronger, the quiet periods are fewer and shorter, and the cycles are largely eliminated (table 1).

Intestinal movements after a cathartic. The administration of castor oil did not change the rate of intestinal movements (table 1).

The effect of psychic influences on intestinal movements. A large number of experiments was undertaken to study the effect of psychic influences on movements of the loops. I found that the movements were not affected nearly so markedly as I had expected from the literature. The sight or smell of food rarely caused a definite increase in movements in an animal which had fasted forty-eight hours. Likewise, a mild reprimand, sudden noise, and minor disturbances had no effect on the movements. If the animal were severely reprimanded, or if a cat were suddenly thrust in a dog's face, there usually occurred inhibition of movements. In no case did I notice an increase in tonus and activity of the loop when gently stroking the dog.

The movements of a denervated loop. In two animals a series of observations was made on the intestinal rhythm, and then the nerves in the mesentery were severed. In one animal the loop was 30 cm. distal to the ligament of Treitz, and in the other it was 30 cm. proximal to the cecum. The rate of rhythmic movements of 17 to 18 in the first animal and of 13 in the second animal did not alter after section of the nerves in the mesentery.

The movements of an isolated loop. The same two dogs were operated on again, and in two stages the loop was isolated from the remainder of the intestinal tract and an end-to-end anastomosis was made to restore the continuity of the tract, the mesentery of the loop was also divided so that all connection between the loop and the abdomen was severed. The loop then derived all of its blood supply from collateral circulation which had developed from the abdominal wall. Records were then made, and it was found that there was a decided decrease in the rate. In the dog with the loop 30 cm. distal to the ligament of Treitz, the pre-operative rate had been

17 to 18 each minute. After isolation, the rate decreased to 12 each minute. Likewise in the animal with the loop 30 cm. proximal to the cecum, in which the rate had been 12 to 14 before isolation, the rate decreased to 8 after complete isolation. As a final check, I isolated a loop which had been used extensively in the making of kymographic records. This loop was 30 cm. distal to the ligament of Treitz, and the rate of the rhythmic contractions, as determined by the kymographic records, was 17 to 18 each minute. After isolation the rate promptly dropped to 13 each minute (fig. 2).

The effect of intravenous injection of solutions of glucose and sodium bicarbonate. The effect of solution of glucose injected intravenously was tried in eleven cases, using from 150 to 300 cc. of 10 per cent solution. In three of the cases some increase in the movements of the loop occurred. In the other eight cases no effect could be noted.

The effect of sodium bicarbonate was also studied. It was given intravenously on six occasions in doses varying from 50 to 200 cc., and in concentration of from 2.5 to 10 per cent. In four of the six cases a marked

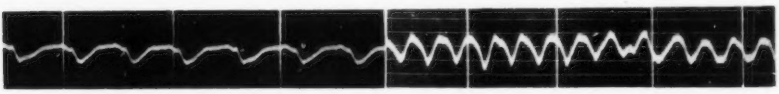

Fig. 2. Electro-enterogram of loop of intestine 30 cm. proximal to cecum. *a*, Taken from the intact loop. *b*, Taken after the loop had been isolated and the mesentery sectioned. Decrease in rate after isolation may be noted.

increase in the intestinal movements, and in the other two cases a slight increase, occurred. Large doses caused the loop to become the seat of extreme activity, the waves sweeping through in rapid succession.

SUMMARY AND CONCLUSIONS

1. There is a definite gradient of rhythmicity in the intact intestine of the dog. The frequency of the rhythmic movements in a loop 30 cm. below the ligament of Treitz is 15 to 16 each minute, and in a loop 30 cm. above the cecum, frequency is 12 to 14 each minute.

2. Fasting, diets of carbohydrate, fat, or protein, or castor oil, did not alter the frequency of these rhythmic contractions.

3. The most common type of movement in the small intestine of the dog is rhythmic, of which different forms exist. In the upper loop the progressive form is most common, whereas in the lower loop the mixing type predominates.

4. Rhythmic movements tend to occur in cycles which alternate with periods of inactivity.

5. The intestine of the fasting dog is relatively much quieter than that of the dog which has eaten recently. Regardless of the site of the loop, the increase in movement after eating is usually prompt.

6. The intravenous administration of solution of sodium bicarbonate has a marked stimulating action on the movements, whereas solution of glucose has a mild, stimulating action.

7. Epinephrine given intravenously promptly stops visible intestinal activity, but has no effect on the electro-enterogram.

8. Isolation of the loop, with section of its mesentery, causes a decided decrease in the frequency of the rhythmic contractions, although the gradient persists.

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REDISTRIBUTIONS OF WATER FOLLOWING TRANSFUSIONS AND INFUSIONS

E. F. ADOLPH, M. J. GERBASI AND M. J. LEPORE

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester

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It is known that when the volume of fluid in the blood stream is suddenly increased much of the extra liquid introduced escapes from circulation. The initial portion of this escape has now been found to occur with great rapidity. The object of the present experiments was to identify the part played by various factors in the initial exchanges of fluids by the blood stream; this was done by varying the composition and the amount of material infused. The sites of exchange were partially identified by measuring changes of concentration not only in the blood but also in several other tissues.

To attain this purpose, sampling of blood and tissues was required at very brief intervals of time. Dogs were used as subjects, anesthetized by a single anesthetic (amytal). Each of the experiments was made as intensive as possible in the hope that the various types of information obtained could be quantitatively correlated. Twenty-one experiments on the same number of dogs constitute the series to be reported.

The investigation started with the transfusion of whole blood from one dog to another. Thereafter the functions of the corpuscular and plasma constituents were successively ascertained by infusing other fluids containing these constituents instead. These included washed corpuscles, serum, sodium chloride in two concentrations, gelatin in three concentrations, and acacia in three concentrations. In this manner the factors of volume, blood pressure, oxygen capacity, osmotic pressure, colloid osmotic pressure, and viscosity can be evaluated from their combined effects.

METHODS. The procedures have been outlined in a previous paper (Adolph, Gerbasi and Lepore, 1933). Sodium amytal was given to dogs intraperitoneally to produce deep anesthesia. It has been already shown that amytal does not promote the escape of plasma proteins through the walls of the capillary blood vessels (Adolph and Gerbasi, 1933). Arterial pressures were recorded either by a mercury manometer or by a membrane manometer connected to one carotid artery. Blood samples were collected from the left brachial artery which had been clamped off and cut. Venous

pressures were recorded by a manometer connected to a jugular vein or to a femoral vein. The trachea was always cannulated.

Blood samples flowed directly from the cut end of the artery into weighing bottles, which were immediately stoppered and placed in moist chambers. Usually three samples were taken at one time, the first of the three being heparinized.

Tissue samples were excised with scissors. These usually included duplicate pieces of muscle, liver, skin, and sometimes intestine. The tissue samples were weighed and then dried at 90° to 100°C. until either constant or minimum weight was reached. Their water contents were calculated as grams of water evaporated per gram of solid residue that remained. These ratios were designated, according to the tissue sampled, *M W*, *L W*, *S W*, *I W*.

Every blood sample was analysed in five ways. These were:

Refractive index of plasma (*P R*) by the dipping refractometer.

Hematocrit (*B T*) by measuring the heights of red corpuscles and of plasma after centrifuging in narrow straight tubes.

Hemoglobin concentration (*B H*) by diluting with water, saturating immediately with carbon monoxide, and comparing with the initial sample in a colorimeter. As no absolute standard was used, it was assumed for certain calculations that the initial concentration of hemoglobin was 13 grams per 100 cc. of amytalized whole blood.

Specific gravity of whole blood (*B G*) by the falling drop method of Barbour and Hamilton (1926).

Water content of whole blood (*B W*) by drying weighed samples in the same manner as tissues.

The change of corpuscle dilution (*B T H*) was calculated by dividing the per cent RBC volume in hematocrit by the per cent hemoglobin.

In five experiments blood and tissues were analysed from chloride, and in three of these for nitrogen contents also. Samples as weighed were digested for nitrogen by the usual Kjeldahl procedure or for chloride by the method of Wilson and Ball (1928).

To facilitate the comparison of apparent volume changes, the water contents of samples were referred not to the wet weights of the samples but to the solid contents. The ratio (*r*) is in all cases the weight of water or the volume of liquid found per unit weight of dry residue or per unit volume of fixed constituent found; the detailed formulae are given in the previous paper (Adolph, Gerbasi and Lepore, 1933). The mean deviations among analyses and among samples are also there indicated.

For nitrogen in tissue the ratio (*M N*) was calculated as

$$\frac{\text{gm. water}}{\text{gm. wet weight}} \times \frac{\text{gm. wet weight}}{\text{gm. nitrogen}}.$$

For chloride ($B\,C$, $L\,C$, $M\,C$) the ratio was

$$\frac{\text{gm. water}}{\text{gm. wet weight}} \times \frac{\text{gm. wet weight}}{\text{millimol Cl}}.$$

The mean deviations in these ratios were small for blood and large for other tissues, as was the case for analyses of dry residues.

All fluids were infused through a cannulated vein. The pressure and hence the rate of infusion was as uniform as it was possible to make it without special apparatus. Usually the fluid flowed into the vein with a gravity pressure of about 30 cm. of fluid, but sometimes compressed-air pressures were used to augment this when the fluid was viscous.

The volumes of fluids introduced into the recipient dogs were accurately known, and samples of the fluids were analysed by the same methods as were all blood samples. The volumes of whole blood in the recipient dogs were always assumed to be 90 cc. per kilogram of body weight (Smith, Arnold, and Whipple, 1921). With respect to each constituent the expected change in composition of the blood was easily calculated. The change actually found was compared with the change expected, to indicate the amounts of fluid apparently gained or lost by the circulating blood. The latter calculation is indicated by the formula:

Volume of whole blood gained as percent of original whole blood volume =

$$\frac{r_2 - r_1}{r_1 + 1} \times 100.$$

Volume of liquid gained as percent of original liquid volume = $\frac{r_2 - r_1}{r_1} \times 100.$

The latter relation was used in plotting all the graphs reproduced. Such estimates of "relative blood volume" are limited in accuracy by the obvious fact that no constituent of the circulating blood is wholly constant in absolute amount. Thus, the corpuscles are being stored differentially in the spleen (and possibly elsewhere) when plethora is produced. But under amytal the spleen is greatly inflated to begin with (Adolph and Gerbasi, 1933), and the amount of such storage is expected to vary chiefly with the volume infused, and not with the composition of the infusion mixture. Any systematic error arising from some removal of corpuscles from active circulation would seemingly not detract from the contrast in the results following the infusion of different mixtures.

The concentration of protein, S, contained in the fluid that leaves or joins the blood may be calculated as:

$$\text{Percent protein in transudate} = \frac{S_1\, r_1 - S_2\, r_2}{r_1 - r_2}$$

The r data most suitable for these comparisons of S values are those for hematocrit ($B\ T$). Little emphasis should be placed upon the values so calculated, since it was impossible to correct accurately for the effect of other solutes upon the refractive indices of plasma from which the percentage values S_1 and S_2 were obtained. The fluid that left or joined the blood that was actively circulating is referred to as transudate, but it is

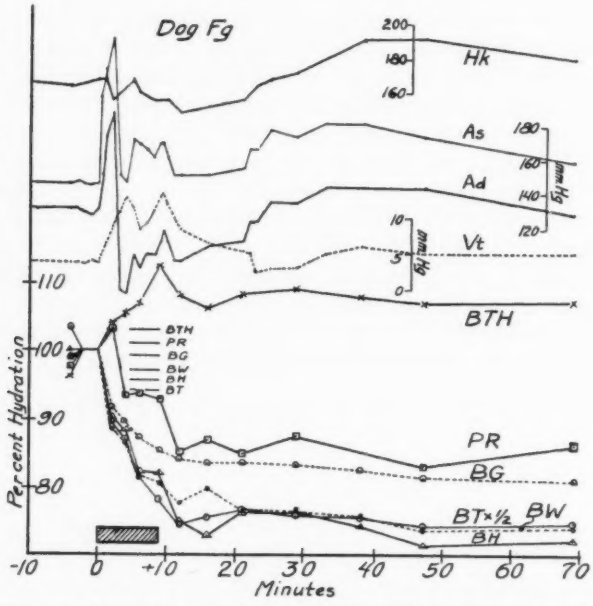

Fig. 1. Changes of circulation and of blood dilution during and after the massive transfusion of whole defibrinated blood (96 cc. per kilogram of body weight) into the left jugular vein. *Hk*, heart rate in beats per minute; *Vt*, venous pressure in the right femoral vein; *As*, systolic pressure, and *Ad*, diastolic pressure in the left carotid artery, as recorded mechanically with a membrane manometer. The other symbols are as explained above. For each measure of blood dilution the change expected when the transfused blood mixed with the recipient's blood is indicated by a horizontal line ending at the time of completion of infusion (9 minutes).

not wholly certain that this fluid went into or came out of extravascular spaces; the possibility must be kept in mind that the fluid was within the blood vessels but not mixed with the streaming blood. Determinations of absolute plasma or corpuscular volume would be of no advantage in this connection, since they too measure only those portions of the blood that are continually mixing with one another at more than some arbitrary minimal rate.

Whole blood. In four experiments (table 1) prepared defibrinated blood, obtained from other dogs, was rapidly infused. In two of these the volume administered equalled the dog's own blood volume; one such experiment is shown in figure 1.

The vascular responses were essentially compensatory to the increased filling of the heart. While the venous pressure rose during the rapid infusion, the arterial pressure soon regulated downward; a part of the compensation was furnished by the decrease of heart rate, with consequent increase of pulse pressure.

The transfused blood was in all respects very nearly equal in concentration to the recipient dog's own blood. The actual results of mixing the two bloods may be compared with the concentrations that would be predicted (table 2). With respect to all constituents the blood rapidly became concentrated. So rapid was the concentration that at the moment the infusion ended the changes in the blood were practically complete. The amount of concentration found indicated that instead of the expected blood volume of 207 per cent, the dog at 21 minutes after the transfusion began had a circulating volume of 164 to 176 per cent, as calculated on the basis of the change in any of the five blood analyses. The plasma lost 350 cc. in 9 minutes and only 35 cc. more at 21 minutes from the beginning of the infusion.

As both plasma and corpuscular concentrations were measured, it is possible to deal separately with the changes in the two. Relative to the plasma proteins, about 16 cc. of water per 100 cc. of plethoric plasma were lost at 21 minutes. This corresponds to 9 cc. per 100 cc. of whole blood. Relative to the hemoglobin, $4\frac{1}{2}$ cc. of water entered 100 cc. of corpuscles or 2 cc. per 100 cc. of whole blood; hence 7 cc. of water per 100 cc. of blood apparently left the circulation. Calculated on the basis of the change in hematocrit this transudate, if such it was, contained 6.7 per cent protein (table 2).

If some of the corpuscles left the circulation and further inflated the spleen, the amount of transudate would be somewhat greater and the protein concentration of the transudate would be slightly lower than was calculated. If axial streaming of corpuscles (Smith, Arnold, and Whipple, 1921) increased after the transfusion, the volume of transudate would be somewhat smaller and the concentration of the transudate would be slightly higher.

In one of the transfusion experiments (Dp) muscle, liver, and skin were analysed for water content, and in another one (De) muscle was analysed. No significant changes were found. It has been explained (Adolph, Gerbasi, and Lepore, 1933) that these changes in order to be clearly significant in a single set of analyses would have to be very large, and also presumably of an orderly character within one experiment.

Washed corpuscles. In four experiments washed corpuscles from other dogs were suspended in 0.9 per cent sodium chloride solution of about normal volume, and infused. In three of these dogs the spleen was extirpated and the blood supply going to the liver was completely tied off just before the infusion. The circulating blood volume was then taken to be 8.3 per cent of the body weight, instead of 9.0 per cent as in the intact animal.

One of the experiments, in which the circulating blood volume was increased 70 per cent by the infusion, is shown in figure 2. In this instance (Du) the fluid supplied was less concentrated in corpuscles and in hemo-

TABLE 1

Summary of transfusion and infusion experiments in dogs under amytal anesthesia

DOG NUMBER	BODY WEIGHT	FLUID INFUSED	VOLUME INFUSED	VOLUME OF PLASMA ADDED TO INITIAL PLASMA VOLUME	TISSUES SAMPLED	DURATION OF INFUSION	RECOVERY FOLLOWED	REMARKS
	kgm.		*cc./ kgm.*	*per cent*		*min.*	*min.*	
Fg	9.7	Whole blood	96	95	*B*	9	60	See fig. 1
Fb	10.0	Whole blood	92	120	*B*	14	52	
Dp	8.9	Whole blood	43	44	*B, L, M, S*	6	43	
De	17.8	Whole blood	18	21	*B, M*	20	60	After hemorrhage
Du	14.6	Washed RBC	58	85	*B, M, S*	11	24	See fig. 2
Dt	26.0	Washed RBC	36	44	*B, M, S*	12	3	Liver clamped
Dr	8.1	Washed RBC			*B, M, S*	12	2	Liver clamped
Dq	13.1	Washed RBC	61	89	*B, L, M, S*	12	44	
Do	13.2	Diluted serum	23	53	*B, L, M, S*	3	34	Plasma depleted
Ek	20.4	0.9% NaCl	49	100	*B, L, M*	12	22	See fig. 3
Dm	20.1	0.9% NaCl	23	52	*B*	11	19	Liver clamped
Em	25.5	1.8% NaCl	39	76	*B, M*	13	26	Did chlorides
Ed	20.0	30% glucose	10	23	*B, L, M, S, I*	5	47	
Ec	25.5	6% gelatin	35	62	*B, L, M*	11	28	See fig. 4
Dn	17.2	18% gelatin	21	44	*B, L, M, S*	18	29	Plasma depleted
Ea	13.3	30% gelatin	11	20	*B, L, M, S, I*	14	27	See fig. 4
En	26.7	6% acacia	51	125	*B, L, M*	26	21	Did chlorides
Ex	19.1	6% acacia	78	156	*B*	20	39	See fig. 5
Ev	25.4	12% acacia	30	71	*B, L, M*	11	26	Did chlorides
Ey	11.1	18% acacia	45	83	*B*	19	36	See fig. 5
Eo	26.0	18% acacia	39	92	*B, M*	16	0	Did chlorides

globin than the dog's own blood. The change of concentration actually found was no greater than that calculated from the compositions of the two fluids. As soon as the infusion stopped, dilution ceased and the blood became progressively concentrated. Evidently the salt solution in which the corpuscles were suspended was partially lost from circulation. By this process the concentration of each constituent returned at least half-way to its original value in the recipient dog. But when the amounts of fluid actually lost are compared with the amounts expected in the mixture of infusion with blood, they are found to be very small. The increase of 65 per cent in circulating volume was evidently able to eliminate fluid from the circulation no faster than the slow rate observed. A partial explanation of

TABLE 2

Transudation of fluid into and from the blood plasma

In each experiment the blood analyzed at the end of infusion was drawn within three minutes of the actual completion.

DOG NUMBER	BT_1 INITIAL	BT_2 AT END OF INFUSION	BT_c EXPECTED	GAIN OF ACTUAL WHOLE BLOOD VOLUME OVER EXPECTED	RATE OF THIS GAIN DURING THE INFUSION	PROTEIN CONCENTRATION OF THE TRANSUDED FLUID	RATE OF GAIN OF VOLUME IN FIRST 10 MINUTES AFTER INFUSION
				per cent	*cc./kgm./min.*	*per cent*	*cc./kgm./min.*
Fg	1.29	0.73	1.14	−19	−3.9	6.7	−0.4
Fb	1.10	0.69	1.32	−27	−3.5	3.5	−0.2
Dp	1.12	0.97	1.07	−5	−0.8	5.0	−0.4
De	1.80	1.80	1.85	−2	−0.1	5.6	−0.2
Du	1.22	1.46	1.46	0	0		−0.7
Dt	1.64	1.48	1.64	−6	−0.6		
Dr	1.18	1.43					
Dq	0.88	1.09	1.13	−2	−0.3	7.9	−1.0
Do	0.92	1.69	1.40	12	2.3	7.5	−1.0
Ek	1.17	2.20	2.34	−4	−0.4	4.3	−1.5
Dm	1.09	2.60					
Em	1.30	2.59	2.29	9	0.6	8.9	−0.6
Ed	0.94	2.36	1.15	56	11.3	0.2	−4.8
Ec	1.73	2.46	2.80	−9	−0.8	10.8	1.1
Dn	1.11	2.16	1.60	21	1.3	1.6	2.4
Ea	1.48	2.38	1.77	22	1.3	2.4	0.4
En	0.96	2.95	2.07	29	1.5	4.2	3.9
Ex	1.26	4.34	3.24	26	2.1	4.4	2.0
Ev	0.84	2.92	1.45	60	6.2	4.2	−0.1
Ey	1.49	5.42	2.74	72	4.8	2.3	−2.4
Eo	0.86						

this fact is found in the progressive fall of the arterial blood pressure after the infusion was completed.

The changes of water content in the tissues were apparently significant in experiment Du (liver and spleen clamped off). The muscle showed a pro-

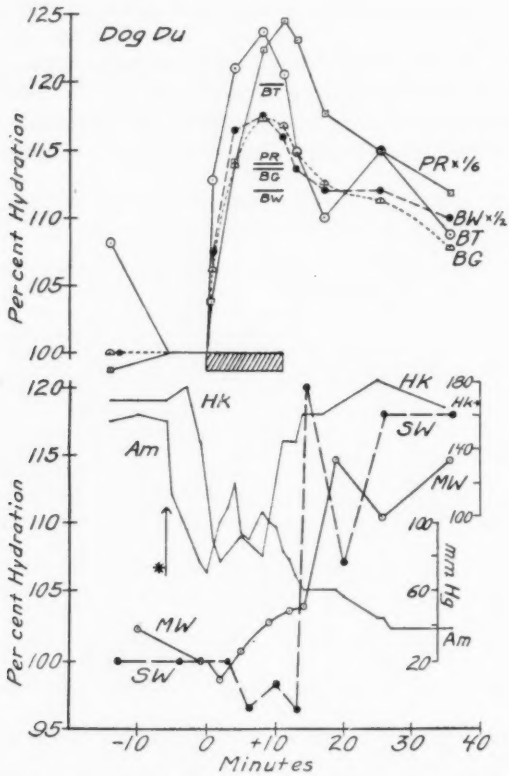

Fig. 2. Changes of blood and tissue dilution during and after the infusion of washed corpuscles suspended in 0.9 per cent sodium chloride solution (58 cc. per kilogram). *Hk*, heart rate in beats per minute; *Am*, mean pressure in the right carotid artery as recorded with a mercury manometer. The other symbols are as explained above. At (*) the liver and spleen were clamped off.

gressive increase of water content up to 15 per cent. The changes in the skin were in the same direction but were not significant, since they were not confirmed in the other three experiments. In one experiment (Dq) the liver was analysed; it showed a progressive increase of water content after the infusion had been completed. In this instance the development of

edema was measured both in the loss of fluid by the blood and in its gain by the liver; meanwhile however the muscular tissue showed no increase of water content.

In two of the four experiments (Dr and Dq) some blood was withdrawn from the dog while the washed corpuscles were being infused (plasmapheresis). It was supposed that greater dilutions of the plasma would thus be obtained, and this proved to be the case. The dilution and transudation did not occur any faster, however, indicating that increase of blood pressure is as important as dilution of the plasma in causing fluid to leave the blood.

The ultimate changes of fluid distribution that occurred after plasmapheresis have been reported by one of us (Lepore, 1932). In none of those experiments was a time shorter than 16 hours allowed to elapse between the plasmapheresis and the sampling of tissues. The experiments here reported indicate that the time at which tissue edema is first produced was not a matter of hours but of minutes. Thus, in experiment Dq, immediately after the infusion of washed corpuscles, the blood was concentrated 1.1 per cent every minute. Hence the rate of transudation amounted to 1.0 cc. per kilogram per minute and this rate prevailed in this experiment for at least 40 minutes. This rate is of the same order of magnitude as the mean transudation rate following severe hemorrhage, which was 0.8 per cent per minute (Adolph, Gerbasi and Lepore, 1933).

The experiments in which washed corpuscles were infused were in two respects not comparable to those in which whole blood was transfused. The amounts given were smaller, and in three of them the liver and spleen were tied off. Both of these factors of difference entered into the realization of distinctly smaller rates of initial transudation.

Serum. It was planned to infuse serum into dogs, to find whether a plethora of plasma was equivalent to a plethora of whole blood. Only one experiment was done, by infusing serum into the same dog from which the serum had been taken 22 and 24 hours previously. On those occasions the corpuscles withdrawn with the serum were promptly returned to the dog's blood stream, suspended in 5 per cent glucose solution. The serum thus obtained was diluted somewhat with 0.9 per cent sodium chloride solution before being infused. During the infusion some fluid was drawn into the circulating blood; directly after the infusion transudation occurred outward, leaving a plasma of almost normal refractive index but a whole blood that was diluted about 20 per cent. No significant changes of water content were found in the tissues analysed.

Evidently in this experiment the concentration of plasma proteins was regulated more quickly than the concentration of corpuscles or the volume of whole blood in circulation. This is shown also by the data of Smith and Dick (1932) who infused rabbits with serum of various concentrations

and noted the progressive modifications of hematocrit and serum protein concentration in blood. Weech, Goetsch and Reeves (1933) noted the immediate influx of water when normal serum was added to the depleted plasma of plasma-protein deficient dogs.

Crystalloid solutions. In three experiments large amounts of 0.9 per cent sodium chloride solution were infused. A nearly complete experiment (Ek) is shown in figure 3.

None of the dilutions found was so great as would be given by that same infusion mixture *in vitro*. This, and the shapes of the dilution curves, indicate that the fluid given had left the blood stream in considerable quantities before the infusion was completed. In 34 minutes about half the excess fluid supplied by the infusion had disappeared from the circulation. The corpuscles were not diluted by the infusion mixture.

Chloride left the blood very rapidly while the infusion was going on; in fact it left more rapidly than the water with which it went into the blood. This fact is readily deduced from calculations of the excessive chloride relative to the excessive water present in the blood at any one time (fig. 3). That infused chloride leaves the blood faster than the infused water was also found by Calvin, Smith and Mendel (1933); it is readily accounted for by the fact that, although the infusion mixture is "isotonic," it is 60 per cent more concentrated in chloride than the water present in the whole blood and 30 per cent more concentrated than the corresponding plasma. The extreme rapidity with which chloride leaves the circulation was noted by Melli and Tasso (1930) who found that during 2 minutes within which rabbits were injected intravenously about 75 per cent of the added chloride left the blood. Similar results were observed in eviscerate rabbits. A slightly slower reduction of vapor tension in the blood of cats into which 8 per cent NaCl solution was injected was measured by Hetherington (1931).

The liver showed no significant increase of water content nor of chloride content except transiently at the completion of the infusion. This moderate transient storage of chloride in the liver could be demonstrated best by calculating the chloride concentration of the slight amount of excessive fluid present. The muscles showed no increase of water content nor of chloride content, whether these contents were taken as relative to the dry matter or to the nitrogen present.

When the liver and spleen were tied off before the infusion (Du), a dilution of the blood greater than was to be expected prevailed during the period of infusion. This corresponds to the finding of Lamson *et al.* (1923) that when the hepatic artery is clamped the dilution of the circulating blood by the injected isotonic sodium chloride endures longer. Further light is thrown upon the rôle of the intestine and liver in holding non-circulating blood by the investigations of Jarisch and Ludwig (1927), Crandall and Roberts (1932) and others.

With the concentration of sodium chloride that was infused double that of the "isotonic" solution (Em), the dilution of the blood was maintained longer. Little extra water was drawn into the blood, but evidently that

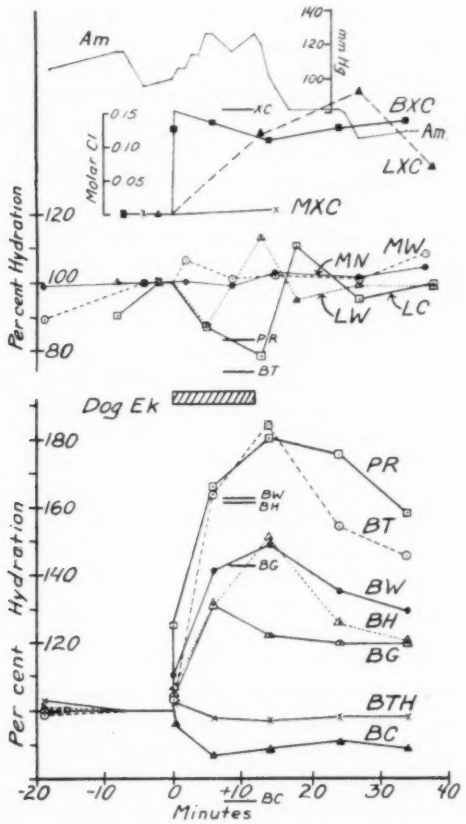

Fig. 3. Changes of blood and tissue compositions during and after the infusion of 0.9 per cent sodium chloride solution (49 cc. per kilogram). Symbols are as in the previous figures; additional are: *BC*, *LC*, relative ratio of water to chloride in blood and in liver; *MN* ratio of water to total nitrogen in muscle; *BXC*, *LXC*, *MXC*, molar concentration of chloride in the extra water found in each kind of tissue.

which was already there did not leave rapidly. Instead, the chloride left the blood very quickly, the concentration present in the excessive fluid of the blood falling 40 per cent by the time the infusion was completed.

Samples of muscle were analysed in this experiment. Only a doubtful

decrease of water content could be found, but the increase of chloride content was significant, whether referred to the dry residue, the nitrogen content, or the water content of the muscle.

One experiment (Ed) was done in which 30 per cent glucose solution was infused into a dog. This solution was about 5½ times the concentration of an "isotonic" solution. An amount of 10 cc. per kilogram caused no toxic symptoms to appear. The solution was less dilute than blood with respect to refractive index, dry weight and specific gravity, yet the infusion immediately caused a great dilution with respect to each of these. Evidently glucose in high concentration was able to attract water into the blood. Somewhat similar results were observed by Brasol (1884), White and Erlanger (1920), Sato (1929), and others. But the water did not remain long, for the relative blood volume decreased with great rapidity, so that in 20 minutes after the infusion was completed the volume was restored from 250 per cent (table 2) to the original 100 per cent (*B T*). Some water was drawn from the corpuscles, being restored to them in the subsequent period of 20 minutes. Four kinds of tissues were analysed, but the only one showing a significant change was liver; this *decreased* its hydration ratio some 30 per cent during the same 20-minute period.

These experiments with solutions of crystalloids indicate that blood volume was rapidly diminished by loss of the extra fluid from the blood. The fluid did not go from the plasma into the corpuscles to any significant extent. The chloride, being more concentrated in the infused fluid than in the plasma, at first left the blood more promptly than the water. Tissue analyses failed to show whither the fluid went from the blood; but the chloride could be identified in liver and in muscle each in one experiment. Glucose in 30 per cent solution caused an enormously rapid shift of water into the blood and immediately out of it again.

Colloidal solutions. Two kinds of colloidal solutions were used, gelatin and acacia. These were made up in varying concentrations. In the case of gelatin, 6, 18, 30 per cent solutions were tested; in all of these 0.9 per cent NaCl was present.

The essential results with gelatin are brought out in figure 4. The 6 per cent gelatin (Ec) diluted the blood relative to all its constituents that were measured. The 18 (Du) and 30 (Ea) per cent solutions concentrated the blood with respect to plasma refractive index (*P R*) but not with respect to whole blood constituents (*B T*). The events after completion of the infusions are of interest. In all cases dilution occurred; in 6 per cent it outran that expected, while in 18 and 30 per cent it overcame the increased concentration of the plasma that the infusion mixture conferred. Obviously water was being taken into the circulating plasma; none of it came from the corpuscles, and in all experiments the liver increased in water content. The other tissues analysed showed no significant changes, so

that it is quite unknown where the water was drawn from into the blood and the liver.

It is obvious that 6 per cent gelatin is of a concentration more than sufficient to keep water from leaving the blood when it is infused. The higher

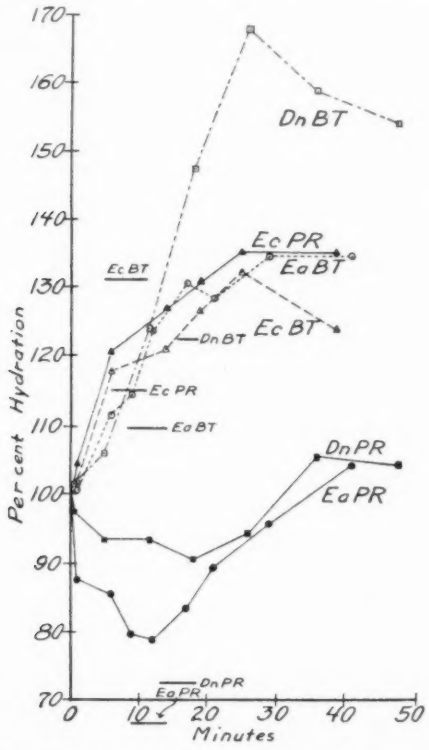

Fig. 4. Changes of blood dilution in three experiments during and after the infusion of gelatin solutions. The amounts and concentrations infused are given in table 1. Two of the measures of dilution are indicated; *PR*, hydration as calculated from the plasma refractive index, and *BT*, hydration as calculated from the hematocrit. *All the data for BT are upon half the scale indicated by the ordinates.* The dilutions expected upon mixing the blood with the infusion mixture are indicated by the horizontal lines whose right hand ends are at the times when the infusions were completed.

concentrations actually drew fluid into the blood, so that the blood volume increased not only because of the added infusion fluid itself but also presumably at the expense of extravascular media. With 30 per cent gelatin (Ea) three times as much dilution of the blood (*B T*) occurred as would be

accounted for by the fluid actually infused. The rate of this influx to the blood was rapid, amounting (fig. 4) to an increase of blood volume of 1.4 per cent per minute; this was about the same rate as the influx that followed extreme hemorrhage (Adolph, Gerbasi and Lepore, 1933).

The results with acacia are indicated in figure 5. In three of the five experiments (Ex, Ev, Ey) acacia prepared for surgical infusion (Eli Lilly & Co.) was used, and this material maintained the arterial pressures at normal levels.

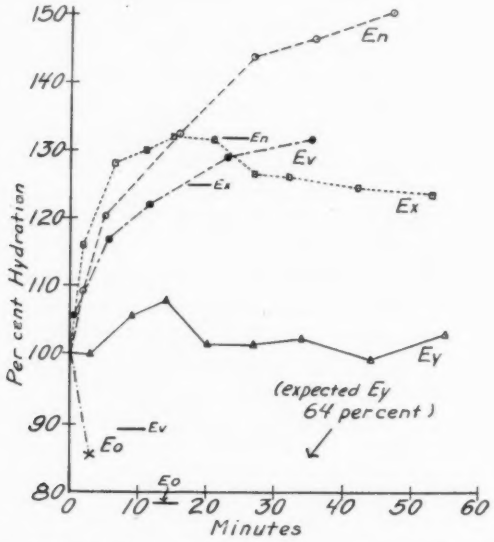

Fig. 5. Changes of blood dilution in five experiments during and after the infusion of acacia solutions. The amounts and concentrations infused are given in Table 1. One measure of dilution is indicated, namely, the hydration as calculated from the plasma refractive index *PR*. The dilutions expected upon mixing the blood with infusion mixture are indicated by the horizontal lines whose ends are at the times when the infusions were completed.

In concentrations as low as 6 per cent (En and Ex), acacia solution attracted water from presumably extravascular sources. The influx was amazingly rapid; a 12 per cent solution containing 1.8 per cent NaCl (Ev) drew inward a volume of 68 cc. of fluid per kilogram of body weight (table 2) before the infusion was completed. It is evident, too, that the concentrated salt was indispensable with the acacia for producing this extreme effect. The 18 per cent solutions of acacia also produced an enormous influx of fluid into the blood, but this influx ceased (Ey) as soon as the infusion was completed, and was followed by a prolonged reconcentration

of the whole blood. This was a relatively slow process and was still under way at the end of one hour.

The acacia infusions produced the most rapid transudations of fluid that were observed. In experiment Ev fluid entered the blood stream at a rate of 6 cc. per kilogram per minute over a period of 11 minutes. In other words, during the infusion a volume of liquid came from the tissue spaces more than equal to the animal's original plasma volume, in spite of the fact that the infusion itself added another 50 per cent to that volume. It is hard to conceive of possible errors or improper assumptions that could destroy the essential validity of this finding; it leads to the view that the whole body holds a mass of mobile fluid, great quantities of which can be translocated almost instantaneously.

In two experiments with acacia (6 and 12 per cent) the liver was analysed, and in both significant increases of water content were found. It is probable that most of this excessive fluid was extra-vascular, and presumably most of it was extra-cellular. In four experiments muscle was analysed, but no significant changes of water content were found. Both muscle and liver increased in chloride content, however.

Attempts were made to find how much the change of blood content might contribute to the change of water content found in tissue samples. So long as the water content of the blood remained essentially unchanged this factor was negligible, because the analyses of dry residue were nearly the same for muscle, liver, and blood. The relations were changed, however, as soon as dilute fluids were added to the blood. In three experiments the hemoglobin was extracted from successive tissue samples and its amount was ascertained colorimetrically. Knowing its concentration in whole blood at the time, the amount of whole blood per unit weight of tissue was calculated. The most extreme dilution of the blood, while tripling the blood content of the tissue, accounted for only 8 per cent increase in hydration (*M W*) of the tissue.

No essential differences were noted in the results of infusing gelatin and of infusing acacia. Similar percentages of each appeared to have similar effects in drawing water into the circulation from extravascular sources. At the same time chloride was given out into liver, muscle, and probably most other tissues.

SUMMARY AND COMMENT. Having mentioned the outcomes of each type of experiment in which fluid was added to the circulation, the results may now be viewed from the standpoint of the separate tissues. Addition of fluid to the blood was merely the means of starting a chain of events in which water was translocated in the body.

The blood itself underwent rapid concentration whenever the infusion fluid was more dilute than the blood with respect to colloids, even though the crystalloids were highly concentrated. When the infusion fluid was

blood itself a similar concentration occurred. But when the fluid exerted colloid osmotic pressure that was to the slightest degree greater than that of the plasma, as 6 per cent gelatin or acacia did, then water was attracted into the blood stream and kept there for at least as long as the one hour's duration of the experiments.

The rates with which the water entered the blood or left the blood were initially very rapid. Their magnitude was as though the filtering capacity of the capillary bed were extremely large (table 2) and capable of transferring fluid rapidly whenever effective forces exerted themselves. The most rapid translocation was produced by infusing 30 per cent glucose solution, but this was also the least permanent of the translocations observed. There is little doubt that apparent transudation *into* the blood stream can be as rapid as apparent transudation *from* the blood stream.

The fixed tissues were analysed in the effort to find where the fluid was exchanged with the blood. In the transfusion experiments the changes of water content found in them were insignificant compared to the large variations among samples of these tissues. But when large amounts of sodium chloride solutions were introduced into the blood, then extra water could be identified as progressively increasing in liver, and in one or two experiments in muscle also. The liver became hard and turgid during infusion of any sort, as has frequently been reported (Cohnheim and Lichtheim, 1877). In the instances where water was drawn into the circulating blood, dehydrated tissues were, with one exception (Ed), not found.

So far as the evidence goes, the immediate exchanges of water following infusions occur pretty generally over the body. This is borne out in a broad way by the results of other investigators, as the oft-quoted studies of Engels (1904) on dogs. The recorded values for changes of water content have sometimes been thought to be quantitatively significant to the extent of permitting estimations, such as have been attempted by others, of the contribution of each tissue to the shift of water balance, but in our experiments over short periods of time quantitative conclusions of this sort were not justified. The nearest approach to our rapid biopsy method of obtaining tissue samples at brief intervals of time was made in the experiments of Tashiro (1926), Baer (1926), and Glass (1928) on rabbit muscle, and of Skelton (1927) on cat muscle. Their shortest intervals of time between infusion (or other experimental procedure) and sampling were 30 minutes. Superficially their results appear to be clear-cut and significant; yet our experiments do not show, except occasionally, a regular progression of changes in water content in dog tissues. An average difference of water content is of value for certain purposes, but a progressive difference in a single experiment is more convincing.

There is, however, some evidence that in the few minutes during and after a massive infusion, water is successively moved first to liver and later to

muscle, later still to other sites of deposition generally known as "the tissue spaces." This is partially illustrated in the protocol of figure 3. Hence it is possible to picture a sequence of movements of water within the body, first to liver, then to muscle, later (Cohnheim and Lichtheim, 1877) to the body cavities and intestine, and finally to the kidneys. There may be other steps in the sequence, and the steps may overlap considerably, but the successive sites represent roughly the immediacy with which the diverse "depots" are utilized.

That the kidneys are unimportant initially in disposing of the extra fluid placed in the blood by infusion has often been ascertained, as by Chanutin, Smith and Mendel (1924). Their negligible rôle is also indicated by the fact that in the experiments water is gained by the circulating blood as readily as it is lost from the blood.

The factors that were modified by the introduction of fluid into the blood stream were principally: circulating volume, blood pressure (especially in the capillaries), blood oxygen capacity, plasma osmotic pressure, plasma colloid osmotic pressure, and viscosity. In the present experiments the effects of four of these six factors were directly ascertained.

Increase in the circulating blood volume resulted in a loss of fluid and an increase of concentration in the blood. The loss of fluid was less, however, when the arterial blood pressure decreased sharply, and this indicated that the factor of importance was the increase of mean capillary blood pressure as the blood volume augmented. So far as is known, volume makes itself felt in no other way than by causing readjustments in the vascular system. Osmotic pressures exerted by crystalloids, chiefly sodium chloride, were temporary and relatively ineffective unless a colloid, gelatin or acacia, was also present.

Hence for the passage of fluid from the blood stream after an infusion one or another of two chief forces was at work. The effective osmotic pressure was reduced by dilution of the plasma proteins, or the capillary blood pressure was raised by means of the increased blood volume, or both. The second was as important as the first, as can be seen in the experiments with whole blood or with 6 per cent gelatin or acacia solutions, where the first factor did not prevail.

The experiments show that sodium chloride ordinarily passed in and out of the blood stream with perfect facility. The salt apparently passed out of the blood more rapidly than the water passed out with it; it did this even from an "isotonic" (0.9 per cent) concentration. But when the salt was given in higher concentrations with acacia, then it reinforced the effect of the acacia in drawing fluid into the blood from outside of the vascular system.

Estimates were made of the concentrations of protein that were present in the fluid passing out of or into the active blood stream (table 2). These

concentrations turned out to be not only reasonable in values but fairly uniform in magnitudes. Further, the values were the same whether the fluid was leaving the blood or entering it, confirming previous observations upon the result of hemorrhage. There is at present no more direct evidence for such an influx of proteins, and no knowledge concerning the exact source of it. But the influx was previously observed by Scott (1916) after the same infusion procedure, and by Smith, Belt, and Whipple (1920) after extreme plasmapheresis. It may depend upon the movement of considerable fluid inward through the walls of capillary blood-vessels (Field and Drinker, 1931).

The experiments seem to indicate that sodium chloride or glucose was able to exert some osmotic pressure that was effective approximately only so long as it was being added to the blood. Immediately thereafter sufficient crystalloid had escaped so that it no longer drew fluid into the blood from the tissues. The important point at issue is that over short periods of time it is not permissible to identify the effective osmotic pressure of the plasma with the colloid osmotic pressure.

Two other factors, mentioned above, may be of importance: blood oxygen capacity and blood viscosity. Oxygen capacity was essentially unchanged in the transfusion of whole blood and in the infusion of washed corpuscles. Contrasting these on the one hand with the infusion of plasma and of salt solution on the other hand indicates that the corpuscles played no essential rôle with respect to transudation. Sufficient dilution of corpuscles would lead to a lack of oxygen that might influence the rate of water exchange, but there was no evidence that significant anoxemia prevailed in the present experiments.

Viscosity was undoubtedly modified in two ways; first by the decrease of the proportion of corpuscles in the blood, and second by the decrease or increase of plasma viscosity depending upon the fluid infused. The decrease in viscosity when washed corpuscles or salt solution was infused undoubtedly contributed to the fall of arterial blood pressure then noted. The maximum increase in viscosity, which was obtained when 30 per cent gelatin was infused, appeared not to embarrass the circulation severely, and it is doubtful whether the viscosity changes introduced played an essential rôle in the outcome of the experiments. Increased viscosity usually accompanied increased colloid osmotic pressure; these two factors might be expected to oppose one another in promoting the exchange of fluids.

The chief result of the experiments was to measure with accuracy the initial rates at which water leaves or enters the blood stream after infused fluid had upset the fluid equilibria of the dog's body. Readjustments were so rapid that *most* of the passage of fluid across the walls of blood vessels occurred before the infusion was completed (table 2). The rate of entrance

or exit depended upon the *volume* of fluid infused per minute as much as upon the change in plasma colloid osmotic pressure caused by the fluid, except where the concentration of colloidal constituents was very great.

Attention should be drawn also to the regular reversals of the direction of transudation within brief periods of time. When a concentrated solution was infused (table 2), fluid was drawn into the blood stream from the tissues during the infusion, only to leave it again in some instances almost as rapidly as soon as the infusion was completed.

When fluid entered the blood stream, as often occurred, at a rate of 1 cc. per kilogram of body weight per minute, the plasma was gaining in volume at a rate of 2 per cent of its original volume per minute. On the whole infusions were capable of producing much faster fluid exchanges than hemorrhages could.

CONCLUSIONS

1. Fluids in large amounts were infused intravenously in 21 anesthetized dogs. Exchanges of fluid were measured by means of the modifications of concentrations in the blood and in tissues sampled at frequent intervals.

2. Whole defibrinated blood or suspensions of washed corpuscles caused plasma to leave the blood stream very rapidly. The initial rate of apparent plasma transudation depended chiefly upon the volume transfused.

3. Solutions of sodium chloride promptly left the circulating blood in every case. Glucose in high concentration very temporarily drew fluid into the circulation.

4. Gelatin or acacia added to sodium chloride solutions brought about more rapid and more permanent translocations of fluid. In high concentrations large volumes of fluid joined the circulating blood. The extra fluid was identified in liver as well as in blood. Chloride either accompanied the fluid or redistributed itself in apparently equal concentrations between the plasma and the other extracellular fluids.

5. The speed with which translocation of fluid occurred was calculated for each experiment. The highest rate at which water entered the circulating blood was 6 cc. per kilogram of body weight per minute. Most of the movement was completed before the rapid infusion had ended.

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LACTIC ACID METABOLISM OF MUSCLES MADE NON-IRRITABLE BY SUGAR SOLUTIONS

A. H. HEGNAUER

From the Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, New York

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Coincident with the non-irritability produced in muscles by immersion in isotonic sugar solution there is a marked increase in the rate of oxygen consumption. Subsequent addition of traces of NaCl or of a variety of other electrolytes produces an equally sudden and marked fall in oxygen consumption (Fenn, 1931). An adequate explanation of these wide variations in oxygen consumption would help materially in understanding the normal mechanism by which the rate of oxygen consumption of cells is regulated. Meyerhof (1924a) has shown that in many cases the rate of oxygen consumption follows the concentration of lactic acid in the muscle and he believed that lactic acid is the sole pace maker for the oxygen consumption. Although it seemed hardly conceivable that the lactic acid concentration could change rapidly enough to explain these sudden variations in oxygen consumption observed in sugar solutions it was obviously necessary to exclude this explanation before further progress could be made. The lactic acid concentration is also of interest from the point of view of the slight contracture produced in muscles by isotonic sugar. The following experiments were undertaken with these problems in mind.

METHOD. The first experiments were made in 1929 upon freshly isolated frog gastrocnemii. Although resting values for lactic acid were fairly uniform the variations observed in sugar solutions were so marked that it was suspected that the oxygen tension in the interior was inadequate to secure strictly aerobic conditions at all times. It was decided, therefore, to use smaller muscles to overcome this difficulty, and to this end the sartorius, semitendinosus, and ileofibularis muscles were used, the three from one side of the frog yielding enough tissue for accurate lactic acid analysis after treatment with sugar, while those from the other side were used as controls.

Dextrose (3.76 per cent) was chosen for a sugar solution since it is readily removed by $CuSO_4$ and $Ca(OH)_2$ in the analysis.

Lactic acid was estimated by the method described by Friedmann, Cotonio and Shaffer (1927). Analysis of resting muscle at room tempera-

ture yielded values ranging from 0.020 to 0.032 per cent, the average being 0.025 per cent.

The most consistent resting values were obtained on muscles which had been allowed to remain in Ringer's solution in the cold room at 4 to 5°C. for several hours or over night before immersion in the sugar solution. All the data reported in these experiments were obtained from muscles so treated.

After the period in the low-temperature room one set of three muscles (sartorius, ileofibularis and semitendinosus) was removed from the Ringer's solution and placed in 5 cc. isotonic dextrose saturated with oxygen or purified nitrogen while their mates, from the other leg of the same frog, remained in Ringer's solution saturated with oxygen at the same temperature as those in sugar (23°C.) to serve as controls. After periods ranging from ½ to 5 hours, both sets of muscles were ground with sand in cold trichloracetic acid and subsequently analyzed for lactic acid together with the solutions in which they had been immersed.

Oxygen measurements were made under the same conditions by means of the Thunberg differential volumeter (cf. Fenn, 1927).

Irritability was measured in terms of the resistance in the primary circuit of an inductorium when the potential was 3 volts, and the coil distance was 0 cm.

Results. In figure 1 is shown the course of the lactic acid changes in muscles bathed in isotonic dextrose at 23°C. under aerobic and anaerobic conditions. In the aerobic experiments, the increase in lactic acid continues for 2 to 3 hours when a plateau is reached indicating a steady state of lactic acid formation and removal. The amount of increase is comparable to that observed in KCl contractures or caffeine contractures before the onset of rigor. The variation in the values is extremely great, due presumably to lack of uniformity of the muscles. The "high" and "low" values are indicated by two separate graphs in figure 1 and are probably correlated with similar high and low values found for the oxygen consumption of other muscles similarly treated. In this respect the muscles seem to fall into two more or less distinct groups. Thus in some muscles in sugar the oxygen consumption was increased ten-fold at which level it might continue for several hours (see also Fenn, 1931), while in other muscles the rate of oxygen consumption did not mount as high and returned to the resting level after 1.5 to 2 hours. The course of oxygen consumption in these latter cases is similar to that found after addition of a small quantity of NaCl to the sugar solution. It was also observed that when the rate of oxygen consumption was low the muscles partially retained their irritability. Presumably they accumulated less lactic acid also.

These low values are probably to be regarded for some reason as anoma-

lous, the more usual and typical response being a high lactic acid content and a high oxygen consumption. For this reason the "high" values were used in subsequent calculations.

To determine the amount of lactic acid removed by the oxygen consumed in a given period of time in sugar another series of lactic acid measurements was made under strictly anaerobic conditions, purified nitrogen or hydrogen being used to replace oxygen. The results of these experiments are also plotted in figure 1. These results do not divide themselves readily into a "high" and a "low" group and the total scattering of points along the curve is much less marked than is the case in the aerobic curve. The

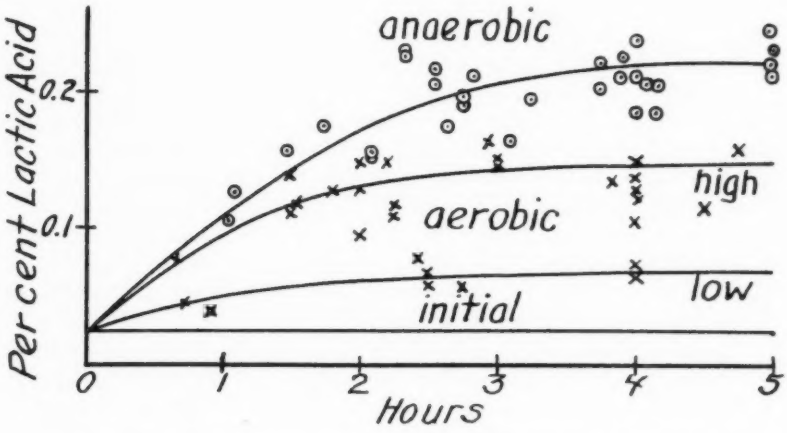


Fig. 1. Lactic acid in muscles (plus solution) after immersion for varying times (abscissae) in isotonic dextrose at 23°C. Ordinates—grams lactic acid per 100 grams wet weight of muscle.

lactic acid which may have diffused out of the muscles into the sugar solutions is included in the figures plotted.

At any time after immersing the muscles in sugar, the difference in height between the anaerobic and the (high) aerobic curve (fig. 1) represents the average amount of lactic acid removed (or prevented from appearing) by the oxygen consumed during the same interval.

In table 1, series A, will be found the figures for the average oxygen consumption for each hour during a four-hour period of immersion in isotonic sugar solution. In the third column are presented the oxygen equivalents of the lactic acid removed during the first, second, third and fourth hours (i.e., the oxygen necessary for complete burning). Columns 4 and 5 give the total oxygen consumed and the total lactic acid removed for 1-, 2-, 3-, or 4-hour periods. The figures are all calculated per gram of

muscle. The oxygen measurements represent averages from nine experiments made on corresponding muscles all of which were found to be non-irritable after removal from the respirometer and were therefore presumably comparable with muscles showing "high" lactic acid concentrations. If this comparison is justified then the rate of oxygen consumption is some-

TABLE 1

Oxygen consumption and lactic acid removed in isotonic dextrose (23°C.)

HOURS	O_2 CONSUMED PER HOUR	LACTIC ACID* REMOVED PER HOUR	TOTAL O_2 CONSUMED	TOTAL LACTIC* ACID REMOVED	OXIDATIVE QUOTIENT
		Series A. Average values			
	cu.mm.	*cu.mm.*	*cu.mm.*	*cu.mm.*	
1	200	135	200	135	0.68
2	190	185	390	320	0.82
3	250	180	640	500	0.78
4	210	75	850	575	0.68
		Series B. Simultaneous measurement on same muscles			
1	265	530	265		
2	325		590	530	0.90
3	402	215	992	745	0.75
4	438	115	1430	860	0.60
		Series C. After addition of NaCl (final concentration = 0.046 per cent)			
0.5	200	130	100	65	0.65
1.5	105	290	205	355	1.73
2.5	40	225	245	580	2.37
3.5	35	100	280	680	2.43
4.0	20	30	290	695	2.40
		Series D. After addition of NaCl (final concentration = 0.092 per cent)			
0.5	215	150	108	75	0.69
1.5	100	335	208	410	1.97
2.5	30	130	233	540	2.32
3.5	25	35	258	590	2.29
4.0	17	25	275	615	2.24

* In cubic millimeters oxygen needed for combustion.

what greater than is necessary to account for the burning of all the lactic acid removed and the oxidative quotient,

$$\frac{O_2 \text{ to burn lactic acid removed}}{O_2 \text{ consumed}} = 0.68 \text{ to } 0.82$$

as shown in the last column (table 1).

In order to establish more exactly the relation between the lactic acid production and the oxygen consumption in muscles in sugar solutions both measurements were made on the same group of muscles. The oxygen consumption (of a group of five or six muscles) was measured for a certain period in sugar after which the muscles (plus the solution) were analysed for lactic acid. Four such experiments were performed the results of which are summarized in table 1, series B. The anaerobic curve in figure 1 is used as the measure of anaerobic lactic acid formation in the calculation of the lactic acid removed during the respective periods. The same relation between lactic acid removal and oxygen consumption is evident in these experiments, the oxidative quotient being 0.90 during the first 2 hours and falling progressively to 0.6 after four hours. It may be observed in the experiments of series B that oxygen consumption is much greater throughout the period of observation, and that the maximum lactic acid concentration under aerobic conditions is less than the average in the earlier series, and remains fairly constant after the first hour and a half. In all instances the muscles were found to be non-irritable, even after one-half hour. Unlike series A, series B did not show any anomalous low values for lactic acid, probably because a larger number of muscles was used for each analysis so that aberrant individual values were averaged out.

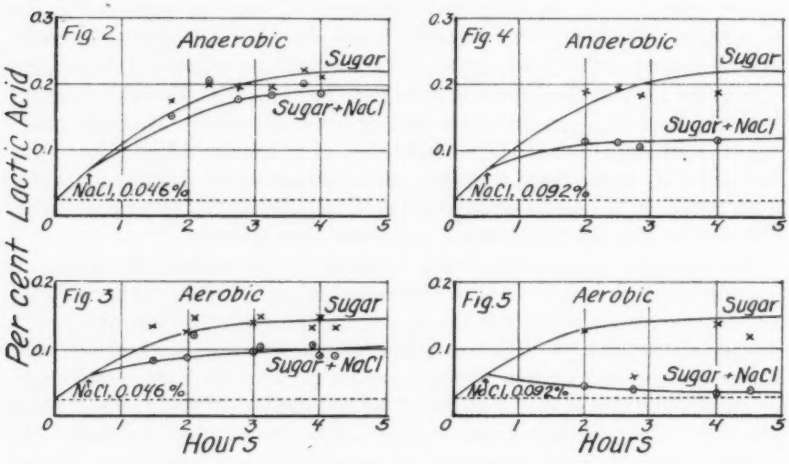
The analyses just described (fig. 1) show that immersion of muscles in sugar solution does in fact cause an increase in the lactic acid concentration within the muscle which might explain, qualitatively at least, the rise in oxygen consumption.[1] The low oxidative quotient suggests however that some of the excess oxygen was not associated with the lactic acid cycle. It remains to describe the effects of adding traces of NaCl to the sugar solution. This decreases the oxygen consumption very suddenly but analyses show that the lactic acid concentration (in solution plus muscle) may continue to increase.

The experimental procedure is the same as before except for the addition of NaCl to the experimental muscles 30 minutes after immersing the muscles in the pure sugar solution. At intervals after the addition both the experimental and the control muscles were analysed for lactic acid. One series was performed under anaerobic conditions and one in the presence of oxygen. The results of the anaerobic series are plotted in figure 2. The upper curve in the figure is made identical with the anaerobic curve of figure 1 and the crosses are the actual experimental results obtained in this series on similar control muscles in pure sugar. When NaCl is added to the sugar to give a final concentration of 0.046 per cent, the lactic acid

[1] It is assumed that the changes in total lactic acid in solution plus muscle are indicative of changes in lactic acid concentration within the muscle itself. Since the sugar solution was unbuffered this assumption is probably sufficiently true for purposes of correlating oxygen consumption with lactic acid content.

values, represented by circles, are (with one exception) slightly depressed below the figures for the controls. The lower curve in the figure is placed below the upper curve by an amount which represents the average difference between the two sets of values. Both curves are therefore somewhat arbitrary but nevertheless represent the facts fairly accurately.

Under aerobic conditions the decrease in lactic acid content caused by the addition of NaCl is more marked and perhaps more uniform than in the anaerobic experiments as is shown in figure 3. The same liberty was taken in the construction of these curves as was exercised in figure 2, the upper curve in figure 3 being identical with the "high" aerobic curve in figure 1.



Figs. 2 to 5. Aerobic and anaerobic lactic acid contents of paired muscles in isotonic dextrose solutions. NaCl was added to one muscle of each pair (circles) at the point marked by an arrow, the final concentration of NaCl being 0.046 or 0.092 per cent. The control muscles were left in pure dextrose (crosses). For method of drawing curves see text.

Oxygen consumption measurements were made on single muscles under these conditions, i.e., in sugar with the addition of NaCl after 30 minutes. In a typical experiment the rate of oxygen consumption is reduced by NaCl from 5.0 to 0.5 cu. mm. per gram per minute as previously described (Fenn, 1931). Averaging a number of such experiments and calculating again the relation between lactic acid removed (difference between lower curve of fig. 2 and lower curve of fig. 3) and oxygen consumed one obtains the results shown in table 1, series C. It will be observed that the addition of NaCl, even in traces, depresses the high oxygen consumption stimulated by pure sugar from 200 (or 400) to 20 cu. mm. per gram per hour and that

it increases the oxidative quotient from 0.64 to 2.4 for the 4-hour period. Moreover these muscles in sugar-NaCl are almost invariably found to be irritable even after some hours in the solution.

It is important to note that in this experiment after the addition of NaCl the lactic acid continues to accumulate in the muscle, though at a decreased rate, even though the rate of oxygen consumption is much decreased. Here is a clear case where the *lactic acid concentration is not the sole pacemaker for the oxygen consumption.* Some other explanation must therefore be sought for the oxygen consumption changes.

In three further experiments simultaneous oxygen and lactic acid measurements on the same muscles under these experimental conditions (Sugar + NaCl) gave oxidative quotients of 1.7, 1.0, 0.7, and these values were found to be correlated with the degree of irritability of the muscles at the end of the experiment, the lowest quotient being obtained on muscles which were non-irritable.

Doubling the concentration of NaCl in the sugar solution has no further marked effect on the course of the oxygen consumption or the oxidative quotient, but causes a more marked change in the course of lactic acid production, aerobic and anaerobic. These facts are illustrated in table 1, series D, and figures 4 and 5, which show the course of anaerobic and aerobic lactic acid formation respectively when the concentration of NaCl in the sugar solution is 0.092 per cent. The anaerobic curves show that there is a decreased formation of lactic acid in sugar-NaCl. This may be sufficient to account for the actual diminution in lactic acid content shown in the aerobic curves (fig. 4) after addition of NaCl. Otherwise it would be necessary to suppose that the marked *decrease* in oxygen consumption after NaCl was accompanied by an *increased* oxidative removal of lactic acid. In this concentration of NaCl the muscles always remain irritable. Calculation of the oxidative quotient, too, gives a value of 2.25 as an average. The average value given by Meyerhof (1924b) for the oxidative quotient in resting muscles by a similar method was 2.6 to 15°C. Hence in sugar plus NaCl the values are approximately normal.

Because similar quantities of KCl were found by Fenn (1931) to depress the oxygen consumption of muscles in sugar, when added some hours after the beginning of the measurements (i.e., when the lactic acid concentration is already at a maximum) it was thought that some information might be obtained concerning the nature of the mechanism involved if lactic acid measurements were made on such muscles. In order to work where the differences might be greatest, the KCl was added (final concentration 0.064 per cent) after the first 30 to 40 minutes of immersion, as in those experiments in which NaCl was used. It was found that the anaerobic lactic acid formation was very slightly increased, by an amount comparable to the decrease due to NaCl. The aerobic accumulation, on the other hand,

was *decreased* by about the same amount, so that the oxidative quotient becomes greater than unity. One is therefore led to conclude that KCl in sugar influences the lactic acid forming mechanism in the same direction as does an excess of that salt in any medium, but that its effect on the oxidative mechanism works for increased efficiency in the removal of lactic acid.

Results at low temperatures. It was shown by Cardoso (1931) that in sugar-non-irritability phosphocreatine is hydrolysed in large quantities at room temperature (40 to 50 per cent in a few hours), but that at 0°C. the muscles maintained a normal phosphocreatine content as well as normal irritability.

Measurements of the lactic acid production at 5°C. revealed that glycolysis is also reduced to a minimal level. The anaerobic increase in lactic acid content of muscles in sugar is scarcely measurable and is within the normal variation of resting muscle, if measured over a period of an hour or two. The resting level at this temperature is 0.017 per cent. The increase is linear with time, however, and after 7 hours, has reached a concentration of 0.05 per cent. The aerobic increase is not measurable, and the only evidence of a change comes from measurements of the oxygen consumption. The resting consumption is 0.07 cu. mm. per gram per minute. In sugar the rate increases very quickly to 0.14 cu. mm. where it remains for about 1.5 hours before returning gradually to the resting rate. Apparently at this temperature the oxygen consumption removes the extra lactic acid almost if not actually as fast as it appears. The change in irritability of these muscles is no more marked than that of muscles in Ringer's solution at the same temperature and for equal periods of exposure.[2]

These results seem on the surface to contradict the hypothesis that irritability is controlled wholly by the electrolyte balance across the muscle membrane. The diffusion of electrolytes in physical systems has a very small temperature coefficient and should therefore proceed at much the same rate as at room temperature. But since the system is physiological rather than physical, actual measurements of Na and K migrations should be made at this temperature before conclusions are drawn regarding the phenomenon.

[2] It is probable, however, that the volume of sugar solution in which the muscles are suspended has something to do with the maintenance of irritability. In 5 cc. of sugar solution containing perhaps 0.3 gram of muscle the electrolyte diffusion out of the muscles must produce an appreciable electrolyte concentration in the sugar which would help to maintain irritability. It has been observed in this laboratory that muscles in 100 cc. of dextrose lose irritability completely in one hour even at the low temperatures where the phosphogen is relatively stable (Cardoso, 1931) and little lactic acid appears. Cardoso used only 10 cc. of solution for his muscles.

DISCUSSION. The large increase in oxygen consumption due to sugar has been discussed previously (Fenn, 1931). It is not due to a burning of the sugar as shown partly by the prompt decrease caused by the addition of traces of salt and partly by the fact (Fenn, unpublished) that the R.Q. is not significantly increased (above 0.90) when the muscle is immersed in the sugar solution. It was shown in this paper that during this high rate of oxygen consumption the lactic acid removed or the increased amount which would have appeared in nitrogen was such that it could all have been burned by the oxygen consumed (oxidative quotient < 1). This does not mean however that all this lactic acid *was* actually burned although with an R.Q. of 0.90 and an oxidative quotient of 0.66 it can be shown that this would be just possible if all the remaining oxygen was used for burning fat with an R.Q. of 0.70 (thus: $\frac{0.9 - 0.66}{1.0 - 0.66} = 0.70$). It seems more probable that the oxidative quotient is nearer Meyerhof's value of 4.5 for active muscle with only a small fraction of the total oxygen concerned with lactic acid removal. On account of the low values for the oxidative quotient it is difficult to regard the excess metabolism in sugar as comparable to the excess metabolism of contraction. Thus sugar decreases the oxidative quotient to less than 1.0 while Meyerhof (1924b) has shown that activity increases it from 2.6 to 4.5.

If all of the increased oxygen consumed after immersion in sugar solution is not used for burning and removing lactic acid it would seem necessary to look for something besides lactic acid as the cause of this sudden increase in oxidation. Some other cause is also indicated by the fact that the rate of oxygen consumption can be brought back to normal by traces of salt without a corresponding fall in lactic acid content and in some cases even without interrupting the progressive increase in lactic acid concentration. The effectiveness of NaCl and other electrolytes in this regard suggests that an important factor in the oxygen consumption is the electroyte balance.

SUMMARY

1. Measurements were made of the lactic acid content of muscles at various times after immersion in isotonic dextrose solution both in oxygen and in nitrogen and after the addition of small amounts of NaCl and KCl.

2. The large increase in oxygen consumption of muscles in sugar solutions is qualitatively but not quantitatively explained by the five- to six-fold increase in lactate content since the oxidative quotient is decreased (i.e., in stimulated muscles a smaller increase in oxygen is caused by the same amount of lactic acid).

3. The differences between the aerobic and anaerobic lactic acid contents represent the lactic acid oxidatively "removed." The corresponding oxy-

gen consumed is more than sufficient to burn completely all the lactic acid (oxidative quotient = 0.6 to 0.7).

4. Only a part of the increased oxygen consumed is therefore explained by the lactic acid, some other cause such as the electrolyte balance being probably involved.

5. The addition of NaCl (0.046 per cent) decreases the oxygen consumption to normal values without completely preventing further increase in the lactic acid content. This is further evidence against lactic acid as the sole pacemaker of the oxygen consumption.

6. The oxidative quotient is increased to 2.2 or 2.4 by such addition of NaCl (0.046 to 0.092 per cent). The normal value for resting muscle is 2.6 (Meyerhof).

7. The addition of KCl to the sugar also decreases the oxygen consumption of muscles without a corresponding decrease in lactic acid content, the anaerobic lactic acid formation being even slightly increased. The oxidative quotient however is increased.

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THE EFFECT OF THE THYROTROPIC HORMONE ON AUTO- AND HOMEOTRANSPLANTS OF THE THYROID AND ITS BEARING ON THE QUESTION OF SECRETORY NERVES[1]

DAVID MARINE AND S. H. ROSEN

From the Laboratory Division, Montefiore Hospital, New York

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The fact that the thyroid when autotransplanted into any part of the body shows all of the morphological and chemical changes that are known to occur in the non-transplanted thyroid, and that these changes occur simultaneously and to the same degree (1) has appeared to us to offer sufficient proof that the secretion of iodothyroglobulin is controlled by chemical substances carried in the blood stream rather than by a specific nervous mechanism. It appears established that the excretion of thyroxine is increased by direct massage of the thyroid (Rogoff, 2) and by prolonged electrical stimulation of the cephalic ends of the cervical sympathetic trunks (Rahe, Rogers, Fawcett and Beebe, 3; Haney, 4). (The latter may be a vasomotor effect and therefore indirect massage.)

However, from time to time papers appear claiming to have demonstrated a secretory or regulatory control of the thyroid by the sympathetic nervous system (11) (12).

Recent advances in pituitary physiology and chemistry have provided another means of studying the question of the control of secretory activity in certain organs. Thus, Engle (5) has shown that the implantation of fresh anterior pituitary stimulates follicle ripening and luteinization of homeo-ovarian transplants in male rats. Eitel, Krebs and Loesser (6) have reported the production of hypertrophy of thyroid cells *in vitro* by adding the thyrotropic hormone to the culture medium. Krayer (7) has shown that section of the cervical sympathetic trunk in no way altered the thyroid response to injections of the thyrotropic hormone.

The experiments here reported were carried out on immature guinea pigs to determine to what extent auto- and homeo-thyroid transplants were influenced by the daily injection of the thyrotropic hormone. The principal data are given in table 1.

The preparation of the thyrotropic hormone used was an acetic acid extract of beef anterior pituitary powder (Armour) neutralized and filtered according to the method of Loeb and Bassett (8). As is well known, acetic acid extracts have no demonstrable gonado- or adrenotropic effects. The

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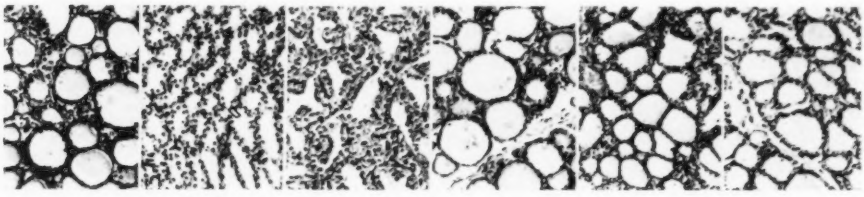
TABLE 1

GUINEA PIG NUMBER	SEX	AGE	WEIGHT	THYROID REMOVED FOR TRANSPLANTATION		THYROTROPIC HORMONE DAILY DOSES (INTRAPERITONEAL)	AGE OF TRANSPLANT	NUMBER OF AUTOTRANSPLANTS	NUMBER OF HOMEOTRANSPLANTS	HISTOLOGY OF AUTOTRANSPLANTS	HISTOLOGY OF HOMEOTRANSPLANTS	HISTOLOGY OF AUTOPSY THYROID
				Amount	Histology							
Series 232												
		days	*grams*				*days*					
16	M.	11	142	½ L. lobe	Normal	1 cc.— 9 days 2 cc.— 4 days	15	1		Mod. marked hypertrophy		Mod. marked hypertrophy
17	M.	11	129	½ L. lobe	Normal	1 cc.— 9 days 2 cc.— 6 days	17	1		Not found		Mod. marked hypertrophy
18	F.	9	137	½ R. lobe	Normal	1 cc.— 9 days 2 cc.— 8 days	19	1		Marked hypertrophy		Marked hypertrophy
19	M.	9	151	½ R. lobe	Normal	1 cc.— 9 days 2 cc.— 7 days	18	1		Mod. marked hypertrophy (Active parathyroid trans.)		Mod. marked hypertrophy
20	F.	9	154	½ L. lobe	Normal	1 cc.— 9 days 2 cc.— 7 days	18	1		Mod. marked hypertrophy		Mod. marked hypertrophy
Series 240												
29	M.	—	256	R. lobe	Normal	2 cc.— 5 days	6	1	1	Nearly absorbed	Nearly absorbed	Mod. marked hypertrophy
31	M.	—	260	L. lobe	Normal	2 cc.— 9 days	10	1	1	Early hypertrophy	Sclerosing	Moderate hypertrophy
32	F.	—	255	L. lobe	Normal	2 cc.—16 days	17	1	1	Normal	Sclerosing	Normal
33	F.	—	225	R. lobe	Normal	2 cc.—14 days	15	1	1	Early hypertrophy	Normal early hypertrophy undergoing absorption	Early hypertrophy
34	M.	—	262	R. lobe	Normal	2 cc.— 7 days	8	1	1	Early mod. hypertrophy	Beginning regression. Early mod. hypertrophy	Early mod. hypertrophy
Series 244												
53	F.	—	202	R. lobe	Normal	2 cc.— 8 days	10	2		Marked hypertrophy of both		Mod. marked hypertrophy
54	M.	—	188	R. lobe	Normal	2 cc.—15 days	17	2		Mod. hypertrophy of both. (Active parathyroid trans.)		Moderate hypertrophy
55	F	—	165	R. lobe	Normal	2 cc.—12 days	14	1		Marked hypertrophy		Marked hypertrophy
56	F.	—	164	R. lobe	Normal	Control	8	1		Normal		Normal
57	M.	—	201	R. lobe	Normal	Control	13	2		Both normal		Normal

daily and total dosage in cubic centimeters of this extract is given in the table. Intraperitoneal injections were begun the day following thyroid transplantation in series 232 and 244 and on the day of transplantation in series 240. All the animals were sacrificed on the day following the last injection. All transplants were made into subcutaneous fascial pockets over the pectoral muscles.

A comparison of the successful transplants with the remainder of the thyroid at autopsy shows a striking similarity in structure between the transplanted and the nontransplanted thyroid in series 232 and 244, despite the handicap due to the time required for vascularization of transplants. Both the transplanted and the nontransplanted thyroid were very hypertrophic as shown in figures 2 and 3, while a specimen of the thyroid taken for transplantation was normal as shown in figure 1.



1 2 3 4 5 6

Guinea pig 16.
Fig. 1. Left lobe as transplanted.
Fig. 2. Autotransplant at autopsy.
Fig. 3. Right lobe at autopsy.
Guinea pig 57.
Fig. 4. Right lobe as transplanted.
Fig. 5. Autotransplant at autopsy.
Fig. 6. Left lobe at autopsy.

The autotransplants and the autopsy thyroid of the two control animals were normal in structure after 8 and 13 days and similar in structure to the pieces transplanted. (See figs. 4, 5 and 6.)

Guinea pig 34 illustrates the stimulating effect of the thyrotropic hormone on homeo-thyroid transplants during the first week. Engle has reported a similar stimulating effect for the gonadotropic hormone upon homeo-ovarian transplants. The thyrotropic hormone, however, does not prevent the destructive process which usually begins in homeo-thyroid transplants about the eighth day.

Guinea pigs 29, 31, 32, 33 and 34 were considerably older (the exact age is not known) than the other guinea pigs and their thyroids were not only less reactive because of age but there is, in addition, evidence confirming the observation of others of the development of a protective

mechanism against thyroid hyperplasia produced by the thyrotropic hormone. It is probable that the effect of increasing age in naturally decreasing the response of the animal to the stimulating action of the thyrotropic hormone is due to the same protective mechanism that develops more rapidly in very young guinea pigs during the period of injection of the hormone. This mechanism is in all probability due to a hormone of suprarenal cortex and gonad origin as we have many times suggested (9), (10). Guinea pigs 29, 34, 31, 33 and 32, as a series, illustrate the progressively increasing protection against thyroid hyperplasia with the increase in the number of injections.

SUMMARY

Evidence is presented showing that the thyrotropic hormone stimulates auto-thyroid transplants in immature guinea pigs to the same degree in the same time as the nontransplanted thyroid. This reaction is brought about by stimulation of the thyroid cells through the blood stream. It is grossly manifest in 2 to 3 days and cannot be associated directly with a nervous mechanism. This interpretation is even more strongly supported by the fact that homeotransplants of the thyroid are stimulated by the thyrotropic hormone (probably as intensely as autotransplants) during their survival period, even though the usual destructive processes of an immunological nature set in about the eighth day.

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THE REMOVAL OF LACTIC ACID FROM THE BODY DURING RECOVERY FROM MUSCULAR EXERCISE

R. MARGARIA[1] AND H. T. EDWARDS

From the Fatigue Laboratory, Harvard University, Boston, Mass.

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In a recent paper by Margaria, Edwards and Dill (1933) it was shown that in man the speed of the disappearance of lactic acid from blood after exercise proceeds much slower than that portion of the oxidative processes of the body which appears to be attributable to recovery. The conclusion was also drawn that lactic acid appears in blood only when muscular activity surpasses a certain limit of intensity. The inference from these considerations appears to be that lactic acid accumulates in the body during exercise only when the supply of oxygen available in the muscles falls below a certain level and accordingly that lactic acid need not be an intermediary product in the combustion of carbohydrate.

This conclusion rests further upon the assumption that lactic acid can diffuse freely and rapidly between muscles and blood with the result that the concentration of lactic acid in the blood is a trustworthy index of the total lactic acid contained in the body. Evidence in favor of this view is presented in the previous paper and the conclusion is further supported by the fact that during the period of recovery variations in the respiratory quotient which appear to be attributable to the previous formation and to the removal of lactic acid have been observed only when an increase in the lactic acid concentration of blood has been demonstrated. Further these variations in the respiratory quotient during the recovery period are greater the greater the increase of concentration of lactic acid in blood. Figure 1 illustrates this relation. It gives the maximum and minimum values of the respiratory quotient for each period of recovery reported in the preceding paper plotted against the concentration of lactic acid in the blood at the beginning of the period of recovery.

To explain the discrepancy between speed of payment of the oxygen debt and speed of removal of lactic acid from the blood, without reference to other mechanisms for contracting oxygen debt than lactic acid formation, A. V. Hill supposed in 1924 that the lactic acid is always formed as a product of muscular contraction and that its removal in the muscles is a

[1] Fellow of the Rockefeller Foundation.

very fast process: and that only in anaerobic conditions does the lactic acid diffuse into the blood, where its removal is slow. It is clear that, if this were the case, the changes in R. Q. during recovery ought to take place at any time that lactic acid is formed in the body independently of whether the lactic acid is present in the blood or in the muscles.

It was desirable in view of the preceding considerations to obtain direct evidence of the variation of the total amount of lactic acid in the body as a function of time during the period of recovery and especially during the first few minutes of this period when our analysis of the data seems to indicate a recovery process with a velocity 30 times that of the rate of removal of

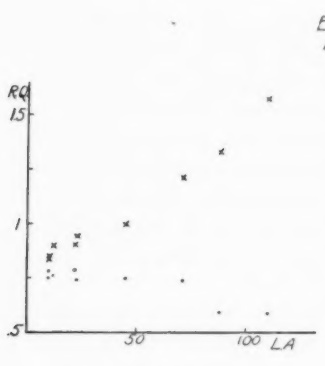

Fig. 1

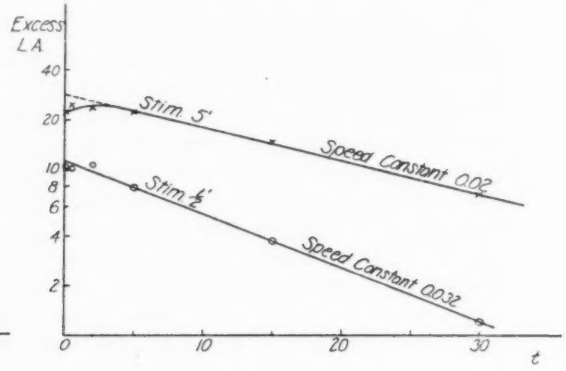

Fig. 2

Fig. 1. Maximum values of R. Q. observed during the first 5 minutes of recovery, x, and minimum values of R. Q. observed at about the 40 minutes to 50 minutes of recovery (.) as functions of the lactic acid concentration of the blood at the beginning of recovery. (Data from Margaria, Edwards, and Dill, 1933.)

Fig. 2. Relative excess concentrations of lactic acid in mice over the rest value, as a function of the time since the end of exercise. The relative value in rest is taken as 10. The values on the ordinate are plotted in a logarithmical scale. Each curve represents the average of 6 experiments given in detail in table 2.

lactic acid in the blood. Assuming that the oxygen consumption during recovery is nearly identical in man and the mouse we have limited our observations to the speed of removal of lactic acid from the body of mice.

METHOD. Young mice of 4 to 8 grams each were exercised until exhaustion by giving them slight electric shocks from an induction coil: the frequency of these shocks was 1 per second, the duration of each about one-fifth of a second. The electrodes were placed on the hind legs so as not to interfere with the respiratory activity. The exercise was due not so much to the muscular contractions derived from the electric stimulation as to the animals' efforts to escape. In fact the frequency of the stimuli over a cer-

tain value had little influence on the amount of lactic acid formed (see table 1). The period of stimulation lasted in two series of experiments respectively $\frac{1}{2}$ a minute and 5 minutes. The lactic acid was measured by the method of Friedman, Cotonio and Schaffer on a trichloracetic filtrate of the whole body after freezing by dropping the mice in CO_2-snow-ether mixture, grinding with sand, and treating by the usual copper-lime procedure.

In each experiment 5 to 7 mice were used, all of the same litter, this being very important for uniformity of results. In nearly all the experiments one of the mice was given no stimulation, its lactic acid content representing the rest value.

The lactic acid values so obtained may have a small error due to lactic acid formation during the freezing process. However, if any lactic acid formation took place in such a short time it must have been very small,

TABLE 1

Lactic acid concentration in mice (mgm. per 100 grams of body weight) after a 5 minute period of stimulation at various frequencies

NUMBER OF SHOCKS PER MINUTE	LACTIC ACID CONCENTRATION		
	1	2	Aver.
0	21.4	31	*26*
2	—	43.7	—
4	45.4	39.6	*42.5*
12	44.5	61.2	*52.8*
48	51.7	56.4	*54*
96	56.3	63.3	*59.8*
108	65.9	52.2	*59.1*

as can be seen by the reliability of the absolute values of the rest controls. Also this error would be much smaller for the higher values of lactic acid at the beginning of recovery, which are the ones more important for the present work.

RESULTS. The results are reported in tables 2 and 3. In figure 2 the "excess" lactic acid is plotted against time of recovery, the lactic acid values being represented on a logarithmical scale. There is no doubt that after a delay at the beginning of recovery the removal of lactic acid is a logarithmical function of time, following the equation

$$y = 10^{a - bt}$$

(y = excess lactic acid; a, b = constants.) The nature of the removal process and the value of the speed constant in the five minute stimulation experiments ($b = 0.02$) is the same as observed in men in the blood lactic acid after an approximately equal period of work, thus giving further sup-

port to the assumption that the lactic acid concentration in blood is proportional to the total amount of lactic acid in the body.

From the data obtained we can certainly deny the existence of a fast removal of lactic acid at the beginning of recovery at the time when the rapid payment of the oxygen debt takes place. The statement that this takes place at the expense of some other mechanism, which does not involve lactic acid formation is thus confirmed. On the contrary the removal of

TABLE 2

Amounts of lactic acid in mice, in milligrams per 100 grams of body weight, at various times of recovery

TIME OF RECOVERY	AFTER A 5 MINUTE PERIOD OF EXERCISE							AFTER A ½ MINUTE PERIOD OF EXERCISE						
	A	B	C	D	E	F	Aver.	A	B	C	D	E	F	Aver.
R	41	29.5	22	16.3	18.5	21.0	*25.3*	—	—	18	22	32	18.5	*24*
10″	112	134	60.9	54.2	68	61.4	*82*	67.5	45	44	46	52.5	39	*49*
30″	121	133	68	64	62	70.5	*87.5*	66	42.5	45	43	55	38.5	*48.5*
2′	131	122	72	65	53	61.5	*85*	65	37	48.5	46	65.5	35.5	*49.5*
5′	116.5	120.5	75.5	49	64	61.5	*82*	49	39	39	34	57	35	*42.5*
15′	91	74	—	46.5	52	50.5	*62*	45	24	31	30.5	42	25.5	*33*
30′	51.5	—	—	—	43.5	30.5	*43*	—	—	25.5	23	33	18.5	*27*

TABLE 3

Speed of formation of lactic acid, under the experimental conditions described in text: amounts of lactic acid in mice at the end of the exercise, after the period of stimulation indicated

Average of 4 experiments

TIME OF STIMULATION	LACTIC ACID, MGM. PER 100 GRAMS BODY WEIGHT
0	*25*
10″	*42*
30″	*52*
1′	*58*
2′	*63*
3′	*69*
4′	*75*
5′	*81*

lactic acid at the beginning of recovery shows a delay of about 4 minutes in the 5 minute stimulation experiments and of about 1 to 2 minutes in the half-minute stimulation experiments.

This delay may be interpreted in the easiest way as a delayed lactic acid production, particularly in view of the increasing lactic acid values up to two minutes from the beginning of recovery. It is not excluded, however, that it may partly be due to slower removal.

From Hartree's (1932) myothermic measurements it appears that in the isolated frog muscle at 20° the delayed lactic acid production after a 5 second tetanus does not last more than a fraction of a minute. From the data of direct measurements of the lactic acid in muscles under about the same experimental conditions, given by Lehnartz (1931), Lundsgaard (1931), Meyerhof and Schultz (1931), it is not possible to construct the true events of the lactic acid appearance. In view of lacking data of direct measurements of lactic acid production, and of the complete difference in the nature of our experiments as compared with those mentioned above, it is not possible to compare the results obtained in the two cases.

The removal of lactic acid from blood in men after work carried out to exhaustion may show a lag of over a quarter of an hour, which is hardly reconcilable with the hypothesis of its slow diffusion from muscles to the blood (see protocol 10 in Margaria, Edwards and Dill's paper (1933)). In view of the results obtained on mice such a lag may be attributed, in part at least, to delayed lactic acid production.

In the recovery after half-minute exercise the removal of lactic acid appears to diminish faster than in the recovery after five minute exercise, the speed constant being 0.032 instead of 0.02. This phenomenon seems to be certain. A higher speed of removal of lactic acid was observed also in man for rates of exercise which did not involve a very high lactic acid concentration in the blood (see the protocols in Margaria, Edwards and Dill's paper). Because of the uncertainty of the result, the phenomenon was not then discussed.

We do not hazard an hypothesis to explain such differences in the rate of removal of lactic acid. A faster removal seems to be related not to the duration of the exercise nor to the intensity, but to the amount of anaerobic exercise or to the total amount of lactic acid formed.

SUMMARY

1. The removal of the total amount of lactic acid in the body of mice, after exercise, is of the same character and of the same speed as the removal of the lactic acid from the blood in man. This is taken as evidence that free diffusion of lactic acid may take place between muscles and blood. Therefore, the lactic acid concentration in the blood may be taken as proportional to the total amount of lactic acid in the body, when an allowance of, at the most, a few minutes is made to account for the rate of diffusion.

2. At the beginning of recovery, at the time when a rapid payment of oxygen debt occurs, no corresponding rapid removal of lactic acid is observed, thus confirming that this fraction of the oxygen debt is not related to the lactic acid mechanism.

3. A delay in the removal of the lactic acid, which lasts 3 to 4 minutes,

after the end of a 5 minute period of exercise to exhaustion, is observed. This is interpreted as delayed lactic acid formation.

4. The speed of removal of lactic acid during recovery appears to be higher the smaller the amount of lactic acid produced during exercise.

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OBSERVATIONS ON LACTIC ACID, TOTAL CO_2, AND pH OF VENOUS BLOOD DURING RECOVERY FROM SEVERE EXERCISE[1]

EDWIN P. LAUG

From the Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia

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The effect of exercise on the acid-base balance in the body has been extensively studied in the papers of Hill, Henderson and Himwich and their associates. The fall in pH, alkali reserve, and the rise in lactic acid have been satisfactorily established as being the most noteworthy effects. Exercise of such a nature that the organism can maintain a steady state has been shown to differentiate very precisely between the trained and untrained. In the most violent bouts of exercise, such as those designed by Barr and Himwich (1, 2, 3) and Dennig et al. (4), lactic acid concentrations were recorded as high as 100 mgm. per cent, and pH changes as great as 0.3. In general, with few exceptions, pH determinations were not made but were calculated from the Henderson-Hasselbalch equation. Actual pH determinations when made, were done colorimetrically on serum or plasma. Some observations of pH of venous blood with the glass electrode (5) have shown that values below 7.0 could be obtained after running 440 yards.

In this paper in addition to pH, some of the other factors of the blood which are disturbed during this form of exercise have been studied.

METHODS USED AND ACCURACY OBTAINABLE. All determinations were made on whole venous blood, obtained by puncture of the vein in the cubital fossa, without stasis. The blood was run directly from the needle into a tonometer over mercury and left in an ice bath until the pH and lactic acid samples were taken. pH was determined at 38°C. by means of a glass electrode (6) with an estimated relative accuracy of 0.02 pH. Lactic acid was determined by the new method of Avery and Hastings (7) employing the manometric apparatus of Van Slyke. Duplicate analyses were always made and checked to within 4.0 mgm. per cent. Total CO_2 was determined in the same apparatus, with duplicates agreeing to within 0.5 volume per cent. Alveolar CO_2 was determined by the Fridericia

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method. The conditions under which this piece of apparatus was used presumably made the results least accurate of all the methods employed. Measurements of blood pressure, pulse rate, and minute volume of respiration were also made.

EXPERIMENTAL. The experiments were performed on a number of untrained, first year medical students. Normal values of pH, lactic acid, and total CO_2 were usually obtained shortly before noon and the subject performed the exercise in the early afternoon on an empty stomach. The procedure was as follows: The runner "warmed up" for about 10 minutes and then was timed in a 440 yard run, around a regulation track, without competition. Immediately after the run, he reclined on a low bench, about 10 inches high, with the arm hanging to the ground, so as to facilitate drawing the blood. "Time" was called when the blood sample had been half drawn. As nearly simultaneous with the blood sample as possible, aveolar CO_2 was obtained by a Fridericia apparatus and air collected in a Douglas bag for a 2 minute period for determination of minute volume. On the other arm systolic blood pressure was measured with a cuff and mercury manometer. Pulse rates were obtained by apex beat. During the recovery period these procedures were repeated.

RESULTS. The most acid pH and the highest lactic acid values did not occur in the samples drawn immediately after exercise, but there was a rapid fall in pH and rise in lactic acid which reached a minimum and maximum respectively between 3 to 6 minutes after cessation of the exercise. In the case of Z., table 1, this was interpreted to mean that although the sample after 17 minutes contained more lactic acid than the sample after 1 minute, presumably a sample obtained within 4 minutes after exercise would have been even more acid than 7.01 and would have had a lactic acid value higher than 150.2 mgm. per cent. Such a condition is shown in the experiment on F., where three samples of blood were drawn.

The level of lactic acid in the blood must represent a dynamic equilibrium between the processes that cause its removal and those that cause it to be added from the active tissues. When the exercise is violent, massive quantities formed in the active muscles in a few seconds must spill over into the blood. Pari passu with this, the mechanisms for lactic acid removal are set into motion but are literally "swamped." Hence it occurs that even shortly after the exercise, the level of lactic acid continues to rise.

The total CO_2 being a partial index of the physiologically available alkali for neutralization, continued to decrease steadily, in all the samples studied. In view of the influx of such large quantities of acid into the blood stream, this might be expected. It has been generally assumed that as the lactic acid is removed during later stages of recovery, alkali freed from combination with it should again be available to bind CO_2, and hence total CO_2 should rise. This, however, was not found to be the case during the periods

TABLE 1

	pH	CHANGE IN pH FROM NORMAL		LACTIC ACID	OBSERVED CHANGE IN LACTIC ACID	TOTAL CO_2	CHANGE IN TOTAL CO_2 FROM NORMAL		pCO_2	ALVEOLAR CO_2	MINUTE VOLUME	SYSTOLIC BLOOD PRESSURE	PULSE RATE
		Observed	Calculated*				Observed	Calculated†					
Subject H., ran 440 yards in 58 seconds													
				mgm. per cent	*mgm. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*	*mm. Hg*	*mm. Hg*	*liters per minute*	*mm. Hg*	*beats per minute*
Normal	7.30			5.2		61.3			68.8	40.7	4.8	105	55
After													
1′58″	7.13	0.17	0.16	107.8	102.6	32.8	28.5	25.6	51.7	32.8	18.7	192	120
10′18″	7.09	0.21	0.22	145.5	140.3	24.5	36.8	36.4	42.7	35.3	16.6	144	112
Subject W., ran 440 yards in 67.5 seconds													
Normal	7.35			5.6		61.1			61.7	(40)		110	
After													
4′24″	6.95	0.40	0.25	163.5	157.9	33.9	27.7	39.5	78.5	30.5		155	
10′45″	7.04	0.31	0.23	150.4	144.8	27.3	33.8	36.2	52.8	30.5			
Subject Z., ran 440 yards in 68.8 seconds													
Normal	7.36			21.8		57.7			56.8	(40)	(5.5)	110	74
After													
1′04″	7.01	0.35	0.17	129.8	108.0	42.3	15.4	27.0	85.8	28.6	12.9	180	136
17′12″	7.15	0.21	0.20	150.2	128.0	22.4	35.3	32.0	34.4		9.3	118	80
Subject F., ran 440 yards in 65 seconds													
Normal	7.36			16.42		51.2			50.5	(40)	(5.5)	110	64
After													
1′27″	7.18	0.18	0.17	133.8	107.4	30.9	20.3	26.9	43.8	29.6	39.2	128	120
5′16″	7.07	0.29	0.21	151.0	134.6	25.5	25.7	33.6	46.3	32.4	25.1	112	112
15′55″	7.15	0.21	0.20	144.0	127.6	21.4	28.8	32.0	32.8	32.4	20.8		112
Subject S., ran 440 yards in 57.8 seconds													
Normal	7.34			15.0		49.4							
After													
6′24″	7.10	0.24	0.23	160.2	145.2								
Subject F., ran 440 yards in 67 seconds													
Normal	7.36			14.9									
After													
7′9″	7.08	0.28	0.25	173.9	159.1								

* Calculated on the assumption that 6.3 mgm. per cent rise in lactic acid causes fall of 0.01 pH unit.

† Calculated on the assumption that 1.0 mgm. per cent rise in lactic acid causes disappearance of bicarbonate, corresponding to 0.25 volume per cent CO_2.

( ) Values were not actually determined, but represent average normals.

observed, for in the experiments on H. and F., the total CO_2 continued to decline in the face of a fall in lactic acid and a rise in pH. This is difficult to explain, and it would be unprofitable to theorize upon this point until additional evidence can be brought to bear. Because of the extreme disturbance of acid base balance, there is a possibility that base in combination with lactate is lost to the body via the kidneys. This has been suggested by Wilson (8).

In the cases of F. and H., during the first 10 minutes of recovery, minute volumes of respiration declined, while lactic acid still rose and pH fell. According to Barr and Himwich (2), the venous blood coming from a comparatively inactive set of muscles, such as the arm, in this case, may, during and after exercise be actually more alkaline than the arterial. If this were so in the cases examined, then presumably the respiratory centre might be bathed in blood even more acid than that examined. According to the theory that respiration is governed by the CO_2 or pH levels in the organism, it might be expected that respiration should be stimulated to even greater activity during early recovery. On the other hand, the assumption that the respiratory centre responds with equal alacrity to a change in pH at 7.4 and 7.10 may be open to question. It is, of course, realized that the pH of the surrounding medium may not be the final word since evidence is accumulating to show that the metabolism of the respiratory centre itself may be a determining factor in respiration.

Van Slyke, Hastings and Neill (9) have estimated the physiologically available alkali to be about 28 m. eq. per liter of blood when the pH changes from 7.4 to 7.0 due to an invading acid other than CO_2. This means that for lactic acid it would require an increase of 6.3 mgm. per 100 ml. blood to cause a drop in pH of 0.01 unit, so long as the CO_2 which is produced when lactic acid reacts with $NaHCO_3$, does not pile up in the blood and cause an acid reaction per se. From the rise of lactic acid in the blood, the change in pH has been calculated. A comparison between observed and calculated pH shows that there is usually a fairly close agreement, except in the experiments on W. and Z. where the calculated venous CO_2 tensions rose above the normal values. Here the acid effect of CO_2 presumably had depressed pH below the value calculated, since the bicarbonate, while almost completely available for neutralizing lactic acid, could but function weakly as a buffer against CO_2. Barr, Himwich and Green (1) have observed that usually there is a decrease in venous blood CO_2 tension after exercise but in one case obtained an increase. It is interesting to note that in their experiment where the CO_2 tension of venous blood rose from 47.2 mm. to 72.5 mm. of mercury, the pH fell from 7.29 to 7.03, which is the lowest value they report. This is in keeping with the findings in these experiments.

Upon calculating $\frac{\Delta\ CO_2}{\Delta\ \text{Lactic acid}}$ there was obtained as a mean from all the values (excepting the two in the experiments which showed an increased venous tension immediately after exercise), a value of 0.24 volume per cent decrease in total CO_2 per 1 mgm. per cent change in lactic acid. Barr, Himwich and Green (1) state that if the reaction between bicarbonate and lactic acid were complete the addition of 1 mgm. lactic acid to 100 ml. blood should cause a disappearance of bicarbonate corresponding to 0.25 volume per cent CO_2. They were not able, however, to show such a definite relationship in their experiments. Bock, Van Caulaert, et al. (10) nevertheless have concluded from the work in their paper and from unpublished results that the relationship between lactic acid and CO_2 is approximately reciprocal. That this reaction between lactic acid and bicarbonate is approximately complete is best shown by comparing the observed and calculated CO_2 columns. It is to be noted again that there is no agreement in the cases where the venous pCO_2 rose after exercise. That a complete reaction between lactic acid and bicarbonate can occur has been doubted by Haggard and Henderson (11) who injected enormous quantities of lactic acid into dogs with suprisingly small changes in blood bicarbonate. However, it must be pointed out that the injections of lactic acid were made over much longer periods of time than those during which the production of lactic acid occurred in vivo in our experiments. A slow addition of lactic acid into the blood does not disturb the acid base balance, since time is given for its removal. This is clearly shown in the experiments of Bock, Dill and Edwards (12) and also of Owles (13). However, when the production of lactic acid is fulminant, complete reaction with the bicarbonate presumably occurs.

Lastly, observation on aveolar CO_2 tension, while admittedly rough, indicates the usual fall shortly after strenuous exercise. This is to be expected since aveolar air is essentially in CO_2 equilibrium with arterial blood, (14) and Barr, Himwich and Green (1) have shown without exception that there is always a drop in arterial CO_2 tension after strenuous exercise.

SUMMARY

1. The pH, total CO_2, and lactic acid have been determined in venous blood during recovery from running 440 yards.

2. The highest lactic acid and lowest pH values were obtained not immediately but within 3 and 6 minutes after exercise.

3. Calculated changes in pH and total CO_2 have been found to agree fairly well with the observed changes, except in those cases where the venous CO_2 tension rose after exercise.

4. Total CO_2 continues to decrease during recovery in the face of a rise in pH and a fall in lactic acid.

The author takes this opportunity to thank the group of first year medical students for their coöperation both in acting as subjects and in helping to obtain some of the data presented.

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THE SECOND PHASE OF THROMBIN ACTION: FIBRIN RESOLUTION[1]

RUBY S. HIROSE

From the Laboratory for Experimental Medicine, University of Cincinnati

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Scores of articles have been written on thrombin and its action in blood clotting, but in nearly all of them thrombin action was considered to have ended with the precipitation of fibrinogen as fibrin. Resolution of clots, which we know occurs quite rapidly in body cavities, was not linked in any way with the processes of coagulation, but was considered as a separate autolysis or digestion. Only in recent years has evidence been put forth tending to show that thrombin action does not end with fibrin formation, but that this is only the first step. The second step consists in a disintegration of the fibrin, with the production of a new protein from the fibrinogen and the release of prothrombin unaltered from its original form. Mills and Ling in 1928 published a preliminary report (1) on this resolution of fibrin, and it was at Doctor Mills' suggestion that a more intensive study of the process was made, with results as set forth here.

EXPERIMENTAL METHODS AND RESULTS. Horse plasma was found the most satisfactory source for obtaining fibrinogen and prothrombin, while cephalin was prepared from fresh sheep brains. The methods previously described by Mills and Ling were followed in making the preparations, the purified fibrinogen and prothrombin obtained from the plasma being redissolved finally in a volume of solvent equal to one-third the amount of plasma from which they were originally derived. The prothrombin obtained had the characteristics of a pseudo-globulin, with a pale blue fluorescence to its solution after thorough dialysis against running tap water. It gave no coagulum on heating to 52° to 56°C., but a heavy one at 72°C. Between 56° and 72°C. a gradual loss of activity accompanied the rise in temperature, with a slowly increasing opalescence to the solution. Even at 72°C. however, some thrombin could still be generated by cephalin and calcium addition. The fibrinogen preparation was sufficiently free of prothrombin to give no clot on standing for two days at 40°C., after the

[1] This work was done during the years 1929–32 under the direction of Drs. C. A. Mills and A. P. Mathews, the manuscript now being edited by them for publication. The thesis from which the present article is prepared was submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry.

addition of optimal amounts of calcium chloride and cephalin. Heating to 56°C. gave a heavy coagulum in the fibrinogen solution.

After preparation of the various clotting factors in active form, they were sterilized, the cephalin and calcium chloride solutions by boiling, and the prothrombin and fibrinogen by passage through a sterile Berkefeld filter. They were kept sterile in the ice box and remained sterile during the various resolution experiments.

The first experiment was designed to verify the previous finding of a gradual loss of fibrin during syneresis. A series of tubes was prepared with identical amounts of fibrinogen and thrombin, the latter being made by activating the prothrombin with cephalin and calcium. Clotting was complete in the tubes in ten minutes. After a half-hour the fibrin was removed from two tubes on a glass rod, washed freely in distilled water, dried at 38°C. for 24 hours and weighed. Fibrin from another two tubes was removed after 24 hours, when retraction had not yet appeared. On the third day there was considerable retraction from the bottom and sides, this gradually increasing to complete resolution by the end of the sixth day, at which time the tube contents were clear and sparkling. Weighing of undissolved fibrin on the various days gave:

After ½ hour	3.6 mgm.; 3.6 mgm.
After 24 hours	3.4 mgm.; 3.5 mgm.
After 3 days	2.9 mgm.; 2.9 mgm.
After 4 days	2.6 mgm.; 2.6 mgm.; 2.4 mgm.
After 5 days	2.3 mgm.; 2.3 mgm.
After 6 days	no fibrin left.

After complete resolution had taken place the clear liquid neither clotted on addition of fresh active thrombin nor when added to a fresh fibrinogen solution. It, therefore, contained no thrombin or fibrinogen. On heating it gave no coagulum at 56°C., the coagulation point of fibrinogen, but did so first at 65°C. At 72°C. a second coagulum was yielded, this being the coagulation temperature for the original prothrombin solution.

In order to study the end products of the fibrin resolution more in detail, a gram of fibrin, made from the purified sterile preparations and dried at 38°C. for a day, was suspended and permitted to dissolve in 225 cc. of 0.1 per cent NaCl solution in a cotton stoppered flask kept in a water bath at 38°C. When solution was complete (after about two weeks), a portion was taken for salt fractionation of the proteins. Half saturation with NaCl gave only a faint opalescence, while complete saturation yielded a heavy precipitate (fraction 1). This was removed by filtration and the filtrate half saturated with $(NH)_2SO_4$ (16 gm. per 100 cc.). A second heavy precipitate came down and was removed (fraction 11). Further $(NH_2)SO_4$ addition yielded nothing.

Fraction 1 above was obtained in roughly the same amount as the fibrino-

gen that went into the fibrin formation, and in all likelihood is this protein in a slightly altered form. It coagulates at 65°C., does not precipitate at half saturation with NaCl, and is not clotted by active thrombin. It no doubt is the same protein as described by Hammarsten (2) and termed by him fibrino-globulin. Fraction II after washing with half saturated (NH_2)-SO_4 solution, dissolving in distilled water and dialysing against tap water, was found to be identical with the original prothrombin solution. When treated with cephalin and calcium, it gave active thrombin exactly as did the original solution. It coagulated at 72°C., the same as originally, and had the same faint blue fluorescence on dialysis against running tap water.

Active prothrombin has been recovered from dried fibrin in the above fashion time after time with apparently little loss in activity or amount. One sample of prothrombin can be used over and over again in producing fibrin, being recovered each time by salting out from the solution after the fibrin has disintegrated. In its reactivation to thrombin, however, one must always add fresh cephalin and calcium. Evidently, in the resolution and liberation of prothrombin, the cephalin and calcium are split off to go with the fibrinogen as the new protein, or else left free in the solution in non-utilizable form. Phosphorus determinations on the original fibrinogen and on the new protein fail to show any significant difference to account for cephalin addition.

Resolution of fibrin in sterile media goes on most rapidly in the presence of oxygen, as in a cotton stoppered flask; removal of free oxygen by bubbling hydrogen through the solution slows the resolution. Carbon dioxide delays the process even more than does hydrogen. This favoring influence of oxygen suggests that the thrombin action on fibrinogen may be quite similar to that of hematoporphyrin, where oxygen is essential and the end product a protein very similar to the one described here (3). Crotalin, in rattle-snake venom, seems to possess a similar action (4).

Purified solutions of fibrinogen, on standing in the ice box for a month or more, often lose in large measure their ability to clot, and in such case it was found that the protein no longer precipitated at half saturation with NaCl, but required nearer complete saturation, and that its heat coagulation temperature had risen from 56° to 65°C. With it, as with the new protein derived from fibrinogen by complete thrombin action, there is found a diminution in carboxyl groups and an increase in amino groups. Whether this has any significance cannot at present be said.

Discussion and conclusions. The resolution of fibrin here described, with the liberation of unaltered prothrombin and a new protein, would seem to be the final stage in thrombin action. Formed from the action of cephalin and calcium upon prothrombin, thrombin first unites with blood fibrinogen and as a result of the union insoluble fibrin is produced. This step in its action is of definite value in the body economy, for thus is

derived the main source of clot to stop bleeding from ruptured vessels. The second step, clot disintegration, becomes equally important at times, when clots are formed in the vascular system or body cavities, and it is this step which is described in this article. Whether all syneresis of clots is due to resolution cannot be said. In the laboratory, however, rabbit blood, freshly drawn and kept at 37°C., has at times been observed to clot and undergo complete liquefaction again within two hours. Such blood no longer contains fibrinogen, although otherwise normal. Ordinarily normal blood takes several days for liquefaction of the clot. There would thus seem to be great variation in the speed of thrombin action in this second phase just as there is in the first.

When tissue extracts in dilute form are injected intravenously, the blood may be completely defibrinated without the appearance of any visible clots (5). May it not be the rapid resolution of the fibrin, as described in this paper, which accounts for the lack of visible clots? In body cavities clot masses also often liquefy with amazing rapidity. Perhaps these results have a definite bearing on the old idea (Nolf) that a continual slow deposition and resolution of fibrin is going on in the capillaries, and that to this the other blood proteins owe their origin as suggested personally by Doctor Mathews. The results given in this paper show the production of a new protein from fibrinogen, a protein with a higher temperature of heat coagulation and requiring greater salt concentration for salting out. It is indeed quite possible that here is a physiologic origin of the plasma proteins.

Although there exists the possibility that a separate proteolytic enzyme accompanies the thrombin in these experiments and brings about the fibrin digestion, yet there is no proof that such is the case, and it remains likely that the resolution described here is due to the completion of thrombin action. The recovery of intact prothrombin time after time and the need of new cephalin and calcium for its activation each time,—these are striking facts which admit of no question.

SUMMARY

Using purified clotting factors in sterile solution, it is demonstrated that

1. The syneresis of clots is in reality a resolution ending in complete liquefaction.

2. Resolution of fibrin yields unchanged prothrombin and a new protein which seems to represent the fibrinogen in an altered state.

3. Liquefied fibrin yields no unchanged fibrinogen and no active thrombin. Nor does it yield free cephalin or calcium capable of activating prothrombin. The prothrombin present requires new cephalin and calcium for its conversion to active thrombin.

4. The possible importance of this final stage of thrombin action is discussed in its bearing on the origin of the plasma proteins.

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DEMONSTRATION OF A QUANTITATIVE RELATION BETWEEN STIMULUS AND RESPONSE IN PSEUDOPREGNANCY IN THE RAT

JOSEPHINE BALL

From the Psychobiological Laboratory, Phipps Psychiatric Clinic, Johns Hopkins Hospital

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Within the past fifteen years a series of experiments has been reported which were designed to discover the stimulus mechanism of pseudopregnancy in the rat. The recent papers by Vogt (1931, 1933) and Haterius (1933) contain certain observations for which no very satisfactory explanation has been offered. Some data incidental to the work on sex behavior of the rat done in this laboratory seem to the writer to be pertinent to the problem and to offer a possible explanation for these results.

Vogt (1931) sought to isolate the hypophysis from nervous stimulation by removing the superior cervical sympathetic ganglia and adjoining interganglionic fibers. She found that after this operation the incidence of pseudopregnancy following glass rod stimulation of the cervix uteri was greatly reduced but that copulation with vasectomized bucks was as effective as ever. She suggested that the vaginal plug might be the cause of this differential reaction.

However, Haterius (1933), in repeating and extending her experiments with essentially the same results, attempted to duplicate at least the mechanical features of the plug by inserting cotton pellets into the vagina after stimulating the cervix artificially. But this did not increase the effectiveness of artificial stimulation. He concluded that sexual excitement was the cause of the greater success of the copulatory stimulus.

Vogt (1933) tested the chemical effects of plugs by injecting extracts of plugs or secretion of seminal vesicles or prostate glands, or by implanting fragments of plugs, and found all these procedures entirely without effect. In this paper she also described another way of differentiating between what she now called "glass rod pseudopregnancy" and "sterile copulation pseudopregnancy." She found that rats which had had vaginal smears taken daily for several weeks were less sensitive to glass rod stimulation than were those whose smears had been taken for only a few days, while this differential sensitivity was not exhibited in response to copulatory stimulation.

It is the purpose of this paper to show that glass rod stimulation is probably different from the sterile copulation stimulus in a quantitative way only. Some observations made by the writer suggest that the rats used by Vogt and Haterius received a considerably stronger stimulus in their mating tests than in the artificial stimulation tests. It is believed that their results can be explained as a difference in quantity of the same stimulus without the necessity of postulating a "psychic factor" (Haterius) or a function of the plug beyond that of mechanical cervical stimulation.

EXPERIMENTAL DATA. In connection with a study of sex behavior in the male rat, 9 females were sterilized by removal of the cervix and 12 by ligation, section, or removal of small parts of the uterine horns. It was expected that the first of these operations, cervisectomy, would prevent not only pregnancy but pseudopregnancy as well, thereby permitting the usual short cycles in the females that were used for testing the sex activity of the males. Since each female could then be used more often, such a result would have made possible a reduction in the size of the colony of females necessary for testing the males. However, cervisectomy did not prevent pseudopregnancy but only reduced its incidence. Injuries to the uterine horns, moreover, had much the same effect.

The animals were mated for brief periods of time, usually from 6 to 15 minutes. Each copulation and each ejaculation was recorded. No mating was permitted except during the tests.

When it was found that the long intervals indicative of pseudopregnancy were occurring in the cervisectomized animals, but that it was not the invariable result of copulation, their records were examined with a view to determining why they reacted at one time and not at another. It was discovered that the animals had invariably become pseudopregnant after they had received two or more plugs in one evening, but only 20 per cent of the 10 times when they had received only one plug and never after copulation that had not been carried to the point of ejaculation, even when intromission had taken place as many as 29 times (table 1). Thus it became apparent that in these animals the amount of stimulation was the factor which determined the frequency with which pseudopregnancy followed mating tests.

The records of the animals with uterine horn injuries but with intact cervices were now examined and found to be approximately the same, i.e., 100 per cent response after two or more plugs, 14 per cent (7 cases) response after one plug, and 12 per cent (34 cases) after copulation without ejaculation (table 1). This response to copulation without ejaculation is probably due to the fact that intromission took place a greater number of times in these cases. In all the cases where pseudopregnancy did not occur after no ejaculation, both in this group and the cervisectomized group, intromission had taken place from 1 to 29 times (median 13), whereas these 4

positive cases followed 2, 30, 41 and 44 intromissions, respectively. Only one of these cases, therefore, cannot be explained on the basis of a greater number of intromissions.

Since these two groups were so similar, both showing this quantitative relationship between amount of stimulus and frequency of response, it seemed desirable to test normal females in a similar manner to see if they also responded only occasionally to limited stimulation. Accordingly, 27 normal females were mated to vasectomized bucks. In this group, instead of 14 per cent and 20 per cent, there was 65 per cent response in the

TABLE 1

Percentage of long and short intervals between heat periods in cervisectomized, sterilized and normal rats after various amounts of copulatory stimulation

OPERATION	NUMBER OF RATS	LENGTH OF INTERVALS BETWEEN HEAT PERIODS									
		After no copulation					After copulation, no plug				
		4-5 days		11-19 days		Total intervals	4-5 days		11-19 days		Total intervals
		No.	%	No.	%		No.	%	No.	%	
Cervisectomy	9	24	100	0	0	24	17	100	0	0	17
Uterine occlusion	12	96	100	0	0	96	30	88	4	12	34
Normal females	27	61	100	0	0	61	30	100	0	0	30

OPERATION	NUMBER OF RATS	LENGTH OF INTERVALS BETWEEN HEAT PERIODS									
		After one plug					After several plugs				
		4-5 days		11-19 days		Total intervals	4-5 days		11-19 days		Total intervals
		No.	%	No.	%		No.	%	No.	%	
Cervisectomy	9	8	80	2	20	10	0	0	6	100	6
Uterine occlusion	12	6	86	1	14	7	0	0	8	100	8
Normal females	27	7	35	13	65	20	—	—	—	—	—

20 cases of a single plug. No pseudopregnancy followed any of the 30 cases of copulation without ejaculation in which intromission took place 1 to 34 times (median 9) (table 1). Since the response to one plug was so much higher than in the operated animals, and since even the operated rats had never failed to respond to two or more plugs, it seemed safe to assume that the normal females would also respond invariably to this increased stimulation, and hence it was thought unnecessary to subject the animals to the stimulus of more than one plug.

The details of these findings are summarized in table 1. In this table the column headed "After no copulation" constitutes the controls. Since all

of the non-copulatory cycles were short, it is safe to consider the animals essentially normal and the long intervals the result of the experimental procedures. In other words, the long intervals are interpreted as true pseudopregnancy, and not due to failure of ovarian activity even though no check was made by laparotomy or autopsy.

Each interval has been treated as a separate case. In no instance have rats been tabulated as stimulable or non-stimulable, since there was no indication that they were so grouped.

Cervisectomy would not, of course, preclude the possibility of nervous stimulation. Such an operation would merely injure the peripheral end of the nerve path, leaving the cut ends embedded in the surrounding tissue. A decrease in response to submaximal stimulation, such as the formation of a single plug, instead of complete repression of the pseudopregnancy response should, therefore, have been expected.

It is more difficult to understand why injury to the uterine horns alone should also depress the response. It might have been due to a pathological condition of the uterus caused by retention of fluid. Marked hydrometra was discovered in several rats at autopsy a year after uterine ligation. However, the tests reported in this paper were made within two months after the operation, during which time the rats gave every appearance of normality, including short and regular cycles. With regard to the possible injury to the nerve supply of the cervix in these operations, it may be stated that in no case was the uterine artery cut or ligated, although the branches supplying an excised section of uterus were always tied off. In general, the nerves in this region follow the blood vessels. No main pathways, therefore, should have been interrupted.

Discussion. It is apparent from the preceding data that there is a quantitative relation between the amount of stimulation and success in producing pseudopregnancy, since delayed oestrus always follows a heat period in which two or more plugs have been formed, it is less apt to follow oestrus in which only one plug has been formed, and it occurs very seldom if the copulation has not resulted in ejaculation. This proportionate effect is found not only in normal animals but also in rats from which the cervix has been removed or the uterus injured, which operations merely reduce the effectiveness of the stimulus.

This quantitative relationship between stimulus and response suggests a possible explanation of the fact discovered by Vogt (1931, 1933) and Haterius (1933) that artificial stimulation of the cervix is less effective than the copulatory stimulus in operated rats and also of the fact that glass rod stimulation differentiates rats accustomed to vaginal smears from those that have not had smears taken (Vogt, 1933). The argument, in outline, is that the glass rod provides a relatively weak stimulus corresponding roughly to the one-plug mating, while their mating tests prob-

ably involved in most cases at least two plugs, shown above to be a much stronger stimulus.

That the glass rod stimulus is approximately equal in effectiveness to one-plug mating may be seen by comparing the percentage of long intervals after one-plug mating in the group of normal rats reported above, with the percentages obtained by various investigators using glass rod stimulation. These one-plug matings gave 65 per cent (20 cases) of long intervals, a figure which is roughly comparable to the 70 per cent (151 cases) found by Long and Evans (1922, calculated from table 26, p. 80) and the 68.8 per cent (45 cases) reported by Meyer, Leonard and Hisaw (1929). Vogt's (1931) 91 per cent of 11 cases is unusual and, in view of the small number of cases, may have been due to chance.

That most of the mating tests used by Vogt and Haterius probably involved the formation of more than one plug, is deduced from the fact that they left the females with males for several hours or all night. From an extended study of sex behavior in rats (unpublished) the writer has found that a healthy young rat is capable of making as many as six plugs in two hours and that he seldom makes less than two unless he has been recently bred. Vasectomized males do not differ noticeably from normal animals in sexual potency (unpublished data). It is, therefore, probable that the females under consideration received more than one plug.

Since several-plug matings induce pseudopregnancy invariably it is evident that such tests constitute too strong a stimulus to discover moderate injuries to the mechanism. To test injuries less severe than total obstruction, mechanical or electrical stimulation must be employed unless copulation is limited, and it was when these less effective stimuli were used that Vogt and Haterius were able to show a reduced percentage of long intervals in their experimental animals.

The fact that Haterius was entirely unable to elicit pseudopregnancy by mechanical stimulation in his operated animals, whereas Vogt attained partial success by the same method, does not vitiate this argument because the former was markedly less successful than Vogt with normal rats also. It is evident that his stimulus was less potent or his rats less responsive.

The evident quantitative relation between the strength of stimulus and frequency of response as here set forth would seem to render Haterius' postulate of a "psychic factor" in copulation superfluous. In order to prove definitely that the greater success of the mating tests was due to another kind of (or at least more profuse) stimulus, it would be necessary first to show that the copulation permitted was equal in stimulus value to artificial stimulation in normal animals, and then to show that artificial stimulation was less effective than that same amount of copulatory activity after operation.

With regard to the differential response of rats much or little subjected to vaginal examination ("smearing") it would seem possible to explain this in much the same way, namely, that long "smearing" renders them less sensitive but that several-plug mating is too strong a stimulus to bring out such a slight change in sensitivity. Only the more delicate test of glass rod stimulation brought it out.[1]

In conclusion, it may be stated that these data serve to point out the necessity of quantitative records of mating tests in such experiments. They also show the need of equating the various kinds of stimuli with respect to their effectiveness in eliciting pseudopregnancy and of choosing a stimulus suitable to the extent of the injury experimentally produced.

SUMMARY

Removal of the cervix uteri of the rat does not prevent pseudopregnancy.

There is a quantitative relationship between the amount of stimulation and the percentage of success in eliciting pseudopregnancy.

The data reported by Vogt (1931, 1933) and Haterius (1933) are analyzed in the light of this quantitative relationship to show that it is not necessary to assume a "psychic factor" in the copulatory stimulation of pseudopregnancy nor any function of the vaginal plug beyond its mechanical stimulation of the cervix in order to explain their results. In the absence of further details, their findings can as well be explained as being due to differences in amounts of the same kind of stimulus

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[1] The writer has found that rats that have not been accustomed to having smears taken probably respond more readily to the relatively delicate stimulus of copulation that is not carried to the point of ejaculation than do those accustomed to smears. In 22 such matings, which were made some time after the data in this paper were collected and in a different laboratory, 7 (31 per cent) delayed the following oestrus although the average number of intromissions was the same as that which was entirely ineffective for the group of normal rats. While the groups may not be strictly comparable, Vogt's findings suggest that the reason for this is that the later group which responded had never been smeared until the week of the tests, whereas the earlier, unresponsive animals had been submitted to the smear-taking routine for several weeks.

The author is indebted to Dr. E. V. McCollum for the use of the rats in his breeding colony in collecting these data.

ON THE ALLEGED PRESENCE OF "HEMOPOIETINE" IN THE BLOOD SERUM OF RABBITS EITHER RENDERED ANEMIC OR SUBJECTED TO LOW PRESSURES

ALBERT S. GORDON AND MAX DUBIN

From Washington Square College, New York University

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Numerous investigations have pointed to the presence of a hemopoietic substance in the serum of animals either made anemic or exposed for some time to low pressures. This substance, unknown in nature and origin, has been stated to exert its regenerative effect most markedly on red cells and has been given the name "hemopoietine."

The first to report such activity of the serum from anemic animals were Carnot and Déflandre (1906). Using rabbits which had been given a single moderate bleeding and killed twenty hours later, they obtained a serum which, when injected into normal rabbits, produced a marked rise in the number of erythrocytes. It was found, however, that serum obtained from rabbits which had undergone frequent bleedings possessed no hemopoietic property. This was ascribed to the depletion of the supply of "hemopoietine" in the body.

Gibelli (1911) who corroborated and extended the work of Carnot and Déflandre, reported that dogs and guinea pigs allowed to starve or made anemic by injections of phenyl hydrazine or by hemorrhage, possess a serum which, when injected into normal animals, increases the number of red cells. The serum of the anemic animals, however, when injected into other anemic animals produced no effect. A mixing of the sera obtained from anemic and normal animals was stated as necessary for any hemopoietic activity. Further corroboratory evidence was produced by Giribaldi (1920) with the serum of rabbits made anemic by injections of glycerine.

Förster (1924) observed that the serum of rabbits subjected to low pressures in a chamber produced an increase in the rate at which the number of erythrocytes returned to normal when injected in small quantities into rabbits rendered anemic by a single bleeding. Further, in contradiction to the earlier work of Carnot and Déflandre and of Gibelli, Förster and Kiss (1925) reported an increase in the rate of regeneration of red cells in anemic animals injected with the serum of animals bled over a long period of time.

Although the presence of "hemopoietine" is accepted in the literature, the circumstances under which it produces its effect are in dispute; moreover, the investigators mentioned above did not have at their disposal the only satisfactory method of determining a true regenerative effect, viz., the reticulocyte index. We have therefore thought it advisable to reinvestigate the entire problem.

MATERIAL AND METHODS. *A. Animals.* Healthy rabbits of both sexes were used for the experiments. In order to accustom them to their new environment, they were kept in separate cages in a well lighted and airy room for about a week before and during the entire period of investigation. The diet was a carefully regulated one of cabbage, carrots, hay, and oats. At no time during the investigation did any of the rabbits show digestive disturbances.

B. Counts. Samples of blood from which the red cell and reticulocyte counts were made were always taken from the heart.[1] Experience has shown it inadvisable to use samples from the marginal vein of the ear, for there is too much variation between duplicate samples.

Red cell counts were made in duplicate, each investigator counting 400 squares on a Levy-Hausser chamber for each sample of blood. Thoma 101 pipettes calibrated by the U. S. Bureau of Standards were used. Those counts which did not agree to within 2½ per cent were rejected, and recounts made until we were satisfied that the figures fell within our limits of accuracy.

The reticulocytes, stained with brilliant cresyl blue, were counted in wet mounts. These counts were also made in duplicate, each investigator counting one thousand red cells, the number of reticulocytes present being expressed as a percentage. (For a discussion of the accuracy of this method, see Ramsey and Warren (1932).)

C. Sera and bleedings. All sera were obtained from the clotted blood of rabbits rendered anemic by cardiac puncture or subjected to low pressures. In the latter case, the animals were placed in a steel chamber at a reduced pressure of 410 mm. Hg. (For details as to the procedure, and construction of the apparatus, see Dubin (1934).) After the anemic animals and the animals exposed to low pressures had reached the desired stage, which could be ascertained from their reticulocyte counts, they were stunned by a blow on the occiput, the carotids severed, the blood collected in centrifuge tubes, and allowed to clot. When the serum was to be used on the same day, it was pipetted off after twenty minutes of rapid centrifugalization. Usually it was used on the following day after having separated from the clot in the refrigerator overnight. Care was taken that the serum contained as little hemoglobin as possible.

[1] Autopsies carried out on several of the animals which had been punctured daily over a period of several weeks revealed no damage to the heart and little or no hemorrhage around it.

The animals to be injected were brought to the various stages of anemia by withdrawals of blood from the heart. The injections were made either intraperitoneally or subcutaneously.

RESULTS. The experiments performed fall into two groups: 1. Those concerned with an attempt to corroborate the work of preceding investigators. 2. Those designed to test for the presence of "hemopoietine" by the use of more modern procedure.

First we attempted to repeat the original experiments of Carnot and Déflandre, who had found enormous increases in red cell numbers occurring in normal rabbits (i.e., in some cases more than a doubling of the count in two days) after the subcutaneous injection of about 10 cc. of serum obtained from animals subjected to a moderate bleeding (30 cc.) and killed approximately a day later. We took three normal rabbits and injected each subcutaneously with 10 cc. of serum obtained from rabbits which had been bled $\frac{1}{4}$ of their blood volume[2] and killed twenty hours later. No significant changes in both the reticulocytes and red cells of all the animals were observed, although counts were made daily for five days after the injection. We have thus been unable to confirm the observations of Carnot and Déflandre. At the same time these results contradict those of Gibelli, who besides attesting to the presence of "hemopoietine," claims that it produces its effect only when the anemic serum containing it is mixed with normal serum in the body.

Förster, who accepts the work of Carnot and Déflandre and of Gibelli, claims that "hemopoietine" also appears in the serum of rabbits subjected to a reduced pressure of 410 mm. Hg for from 24 to 49 hours, and that it produces its effect when injected into animals rendered anemic by a single bleeding corresponding to $\frac{1}{4}$ of their blood volume.[3] One of us (M. D.), however, has shown that no significant alteration of the blood picture takes place when rabbits are exposed to this pressure for a time as short as one or two days. It is only beginning with the fifth day that significant changes in the red cell and reticulocyte counts are observed. Consequently, in repeating Förster's experiments, we made use of the serum of rabbits which had been placed in a tank regulated at 410 mm. Hg for five days. This exposure was found sufficient to raise the red cell count at least 25 per cent and the reticulocyte count to a value between 8 to 10 per cent above normal.

Five rabbits, bled $\frac{1}{4}$ of their total blood volume on a single occasion, were then injected with 10 cc. of five day "low pressure" serum on the day

[2] In all the experiments of this investigation, the blood volume was arbitrarily assumed to be $\frac{1}{15}$ of the body weight.

[3] Förster apparently was not aware of the fact that Gibelli had made the point that "hemopoietine" in anemic serum only produces a marked effect when injected into *normal* animals.

following the bleeding. The results obtained with these animals were compared with those in five uninjected control rabbits,[4] also bled ¼ of their blood volume on a single occasion. A study of the red cell counts, made daily over a period of 10 days in all 10 animals, showed that the rate of regeneration of red cells was not any greater in the injected animals than in the controls; in fact, the cells of one control animal exhibited a greater rate of regeneration at the end of the tenth day than any of the injected animals. It was also noted that the reticulocyte peak, reached approximately on the fifth day of anemia in all the animals, varied from 5.8 per cent to 7.2 per cent in the injected group and from 5.6 per cent to 7.8 per cent in the control group. This is sufficient to indicate that the "low pressure" serum, when given in one injection into rabbits bled ¼ of their blood volume (as Förster did), is without any erythropoietic effect.

At this point it seemed to us more likely that if a substance such as "hemopoietine" actually exists, it would be most concentrated in the serum of anemic animals and of animals subjected to low pressures at that stage when the reticulocyte counts were at or near their maximum. Consequently in the last series of experiments we obtained anemic serum from rabbits which had been bled quantities of blood equal to about twice their total blood volume over a period of three weeks. The animals at the end of this time had developed reticulocyte counts ranging from 14 per cent to 20 per cent. The "low pressure" serum was taken from rabbits subjected to a pressure of 410 mm. Hg for a period of two weeks. Counts taken during this period showed that these animals had maintained a reticulocyte percentage ranging from 10 per cent to 12 per cent for a week up to the time they were killed for serum.

In this set of experiments, five rabbits, given a single bleeding equivalent to ¼ of their blood volume, were injected with anemic serum (7 cc. to 15 cc. daily) over a period of a week, and five others, injections of "low pressure" serum (5 cc. to 10 cc. daily) over a period of five days. In some of the animals the injections were begun on the day following the last bleeding; in others the injections were started shortly before or after the reticulocyte peak following the last bleeding was reached. Even working under these optimal conditions we were unable to verify the presence of "hemopoietine" in anemic and "low pressure" serum. Thus the reticulocyte peaks attained in the controls were as high as those reached in the animals injected with serum (i.e., 6.8 per cent to 8.2 per cent in the control animals against 6.1 per cent to 8.3 per cent in the injected group); likewise the rate of regeneration of red cells in the controls was not significantly different from that noted in the injected animals.

[4] Throughout this investigation the control experiments were carried out at the same time as those for the injected animals. In this way the possible effects of seasonal variation in red cell counts, etc., were avoided.

There still remained the possibility that the animals receiving the injections were not in a sufficiently sensitized state (see Whipple and Robscheit-Robbins, 1925) when blood equivalent to $\frac{1}{4}$ of their total blood volume was withdrawn. Five animals were therefore bled 5/24 of their blood volume on two occasions and three animals $\frac{1}{6}$ of their blood volume three times before daily injections of either anemic or "low pressure" serum over a period of 12 days were begun. Even in these animals, however, the serum was without effect, as could be seen from the slow rate of regeneration of red cells and the failure of the serum to affect the reticulocyte count.

SUMMARY

We have been unable to confirm the experiments of previous investigators who have reported the presence in the serum of anemic animals and animals subjected to low pressures of a substance possessing erythropoietic properties.

A more extended series of experiments shows conclusively that "hemopoietine" is absent from such serum.

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CHANGES IN THE CHEMICAL ELEMENTS OF THE BLOOD OF RABBITS DURING EMOTIONAL EXCITEMENT

H. L. KATZ AND L. B. NICE

From the Department of Physiology, The Ohio State University

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This study was undertaken to determine the changes which occur in the common blood constituents during emotional excitement. Nice and coworkers (1931–1933) have shown that in this state there is an increase in the specific gravity and viscosity of the blood. But so far as we are able to learn no comprehensive study has been made on the effect of emotions on the common blood constituents in the same animals.

Glucose. Eckhard (1903) found sugar in the urine of excited rabbits while Hirsh and Reinback (1913) found that handling of rabbits caused an increase in the blood sugar. Rogoff and Stewart (1924), Blinova (1928), Cannon (1929), Schtscherbakow (1930), Lumley and Nice (1930), and Ohmi (1933) have found that sensory stimulation, as pain, increases the blood sugar and causes glycosuria in experimental animals. Painful stimulation, it is believed, brings about the mobilization of sugar from carbohydrate stores by a reflex via the splanchnics and through the adrenal glands. Extensive reviews of the literature on the relation of carbohydrate metabolism to sensory stimulation and the adrenal glands are given by Fujii (1921), Ohara (1925), Satake (1926), and Hirayama (1927).

A decrease in blood phosphorus is accompanied by a rise in blood sugar. The intravenous injection of phosphate solutions reduces the blood sugar and there is a decreased output of urinary phosphate after sugar ingestion: Fisk (1931), Elias and Weiss (1922) and Messini and Poli (1930).

Francaviglia (1928), Heppe and Schliephake (1931) and Feissinger and Cattan (1933) reported that the spleen plays an important rôle in the regulation of carbohydrate metabolism. Chronic hyperglycemia occurred after splenectomy. The injection of splenic extract lowered blood sugar (Heppe).

Nitrogenous constituents. The evidence for the relation of nitrogenous metabolism to excitement is contradictory. Rakestraw (1921) noted uric acid and blood sugar increases during prolonged muscular strain. Wilson (1932) cited evidence for increased metabolism of food proteins but not in the endogenous metabolism. Dubois and Polonovski (1924) found that the increase in the urea nitrogen in the blood is a result of the influence

of adrenalin and is not due to any kidney effect as Quinquaud (1923) contended. Koel (1932) found an increase in blood creatine and creatinine in strenuous exercise.

The amino acid nitrogen in the blood is remarkably constant except during digestion: Van Slyke and Meyer (1912), Gyorgy and Zunz (1915), Bock (1917) and Okada and Hayashi (1922). Benhamon and Gille (1931) attributed increases in non-protein nitrogen to the function of the spleen and claimed there is a decreased amino acid content in the blood after splenectomy. Adrenalin produced an increase in the amino acids by causing contraction of the spleen.

Cholesterol. Lyons (1931) on excitation of cats produced emotional hypercholesterolemia with an increase in the blood cholesterol ranging from 25 to 30 per cent. Low and Pfeiler (1928) and Mazzeo (1928) obtained cholesterolemia after the injection of adrenalin. Abelous and Soula (1926) reported an increase in the cholesterol content in the venous blood as a result of an electrical stimulation of the spleen, and therefore believes in a "cholesterogenic internal secretion" produced by the spleen. But many workers have reported cholesterolemia after splenectomy: Frenckele and Nekludow (1928), Bugnard (1929) and Pizzini (1931).

Inorganic salts. Collip (1927) found that asphyxia caused an increase in serum and inorganic phosphorus. A stimulation of the sympathetics produced an increase in calcium in Zondek's (1922) and also in Speranskaia's (1930) hands. On the introduction of adrenalin the serum calcium content fell (Billigheimer (1922), Gussardo (1930), and serum phosphorus also decreased, Woringer (1924), Winter and Smith (1923–1929)). There was also a decrease in inorganic phosphorus for days after exercise. Rasekov (1928) reported an increase in hemoglobin, sugar lactic acid and nitrogen in the blood as a result of pain. Mirva (1932) concluded that the spleen is one of the important endocrine organs which controls the calcium level of the blood. Lamelas (1930) concluded that the calcium content of the blood of the cat remains fairly constant in spite of conditions which deprive the organism of its sympathetic nerve impulse in toto or in part or after the injection of adrenalin.

PROCEDURE. In these experiments both male and female rabbits were used. All animals were carefully selected with respect to size and health, and were kept on the usual laboratory diet. The animals were handled daily in order to accustom them to the laboratory routine and manipulation. Throughout these experiments the animals were deprived of food for at least sixteen hours, prior to the beginning of the experiment. This was to exclude the possibility of the effects of digestion and absorption on the chemical constituents of the blood.

The animal under observation was held gently in a supine position without anesthesia, and 10 cc. of blood for testing were secured by cardio-

puncture by means of an hypodermic syringe previously rinsed with a 10 per cent solution of sodium citrate. The blood sample was immediately transferred to an Erlenmeyer flask containing 0.1 cc. of 10 per cent sodium citrate solution previously evaporated to dryness. In the process of securing the "basal" (quiet) sample meticulous care was taken not to excite the animal in handling. If the animal showed signs of excitement during the drawing of the basal blood, this sample was discarded.

After 10 to 15 minutes (in a few cases one hour) the rabbit was fastened to an animal holder and excited by being teased from time to time for a period of about three minutes with a weak Faradic current using a Harvard inductorium. The excited sample was drawn approximately three minutes after the excitatory period. The rabbits, both normal and splenectomized, showed all signs of sympathetic stimulation.

Six cubic centimeters of the sample were diluted with seven volumes of water, and the sodium-tungstate filtrate prepared according to the Folin-Wu (1919) procedure for the determination of glucose, non-protein nitrogen, urea, total and preformed creatinine, uric acid, amino acid nitrogen and chlorides. Inorganic phosphorus and calcium were determined on the trichloro-acetic acid filtrate of 3 cc. of serum. The remainder of the sample was used for the determination of cholesterol and hemoglobin.

We employed the following methods: for glucose, Folin (1929); non-protein nitrogen, Folin-Wu (1919); urea, aeration process using the concentrated jack bean urease of the Arlington Chemical Company; preformed and total creatinine, alkaline picric acid method; uric acid, Folin's direct method (1930); amino acids, Folin (1922); inorganic phosphorus, Youngburg and Youngburg (1930); calcium, colorimetric method of Roe and Kahn (1929); chlorides, Whitehorn (1921); cholesterol, Day and Bollinger (1930); for hemoglobin, the Newcomer method was used.

Results. Table 1 shows the average changes in the concentration of the chemical elements in the blood in the quiet as compared to the excited states in 25 tests on 15 normal rabbits. All blood constituents analyzed invariably show an increase as a result of the stimulation with the exception of amino acid nitrogen and the inorganic salts. On the average glucose increased 25.3 per cent, non-protein nitrogen 11.5, urea 15.1, preformed creatinine 32.7, total creatinine 37.1, uric acid 10.7, cholesterol 21.0, and hemoglobin 11.2 per cent. Inorganic phosphorus shows a decrease of 11.8 per cent. Amino acid nitrogen, calcium and chlorides undergo insignificant changes.

The splenectomized group shows much the same changes in the blood constituents following excitement as the normal rabbits with the exception of hemoglobin which shows an insignificant decrease. Glucose shows an increase of 16.6 per cent, non-protein nitrogen 20.4, urea 24.5, preformed creatinine 26.8, total creatinine 17.1, uric acid 16.1, and cholesterol 12.7.

The inorganic phosphorus decreased 11.0 per cent. Amino acids, calcium and chlorides change insignificantly. The hemoglobin shows a decrease of 3.3 per cent in contrast to 11.2 per cent increase in the normal group. This further points toward the spleen as a reservoir for red blood cells which are expelled into the blood stream in emergency states.

The control experiments, that is, rabbits on which duplicate experiments were made without excitement, are also tabulated in table 1. The per cent differences in the chemical constituents are well within the limits of

TABLE 1

Average changes in the blood of normal, splenectomized and control rabbits in emotional excitement

	TOTAL EXPERIMENTS	GLUCOSE	NON-PROTEIN NITROGEN	UREA	PREFORMED CREATININE	TOTAL CREATININE	URIC ACID	AMINO ACIDS	SERUM INORGANIC PHOSPHORUS	SERUM CALCIUM	CHLORIDES (NaCl)	CHOLESTEROL	HEMOGLOBIN
Normal:													
Quiet	25	101.4	35.7	20.5	1.7	3.9	0.64	11.7	5.1	12.5	492	107.5	12.5
Excited	25	127.3	39.8	23.7	2.3	4.4	0.85	11.6	4.5	12.6	498	130.1	13.9
Per cent difference		+	+	+	+	+	+	−	−	+	+	+	+
		25.3	11.5	15.1	32.7	37.1	10.7	0.86	11.8	0.8	1.2	21.0	11.2
Splenectomized:													
Quiet	6	101.9	31.5	16.3	1.8	3.2	0.61	10.4	4.9	14.9	494.5	134.3	13.2
Excited	6	118.8	37.5	20.3	2.3	3.8	0.70	10.2	4.4	14.8	499.7	151.0	12.8
Per cent difference		+	+	+	+	+	+	−	−	−	+	+	−
		16.6	20.4	24.5	26.8	17.1	16.1	1.9	11.0	0.8	1.1	12.7	3.3
Control:													
First test	8	104.9	40.1	26.1	1.3	3.2	0.56	10.8	5.3	13.6	52.0	90.9	12.4
Second test (no excitement)	8	105.7	40.3	26.2	1.3	3.3	0.53	10.7	5.2	13.5	52.4	94.7	12.7
Per cent difference		+	+	+		+	−	−	−	−	+	+	+
		0.7	0.5	0.4	0	3.1	5.3	0.9	1.3	0.7	0.7	4.2	2.4

experimental error. This indicates that the changes in the blood constituents in the normal and splenectomized rabbits actually produced as a result of the excitement (fear, anger, and pain) and are not due to any extraneous factors, for all conditions were identical with the exception of the omission of the electrical stimulation in the control group.

The magnitude of change in the chemical constituents in the normal and splenectomized groups is not the same (fig. 1). The normal group averages larger per cent increases in glucose, preformed creatinine, total

creatinine, cholesterol, and hemoglobin; whereas the splenectomized group reacted to the excitement with larger per cent increases in non-protein nitrogen, urea, and uric acid. The degree of variation in the inorganic phosphorus is practically the same in both groups.

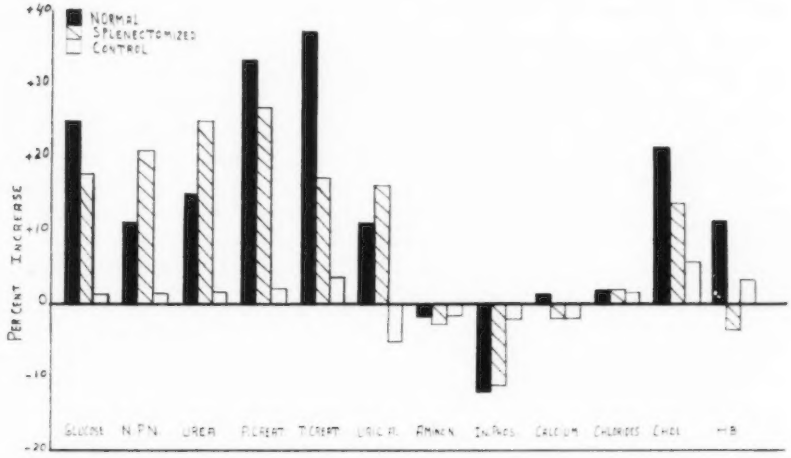

Fig. 1

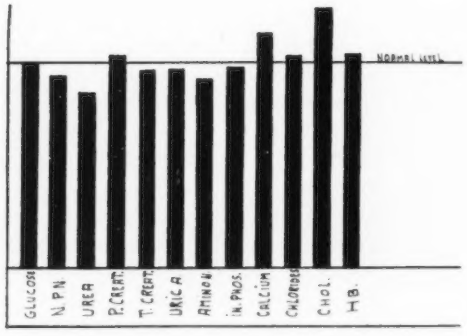

Fig. 2

These facts point to a relationship existing between the spleen and the enumerated chemical constituents of the blood. This relationship and presumed splenic function is not well established, and the mechanism of the action in this respect is not understood. The results, however, indicate that the spleen exerts an inhibitory action on the breakdown of tissue

products which results in an increased concentration in the non-protein nitrogen and urea of the blood; and an accelerating effect on those processes which results in an increased concentration in glucose, preformed creatinine, total creatinine, cholesterol, and hemoglobin.

Figure 2 illustrates the level of the blood constituents in the quiet state following splenectomy in contrast to the level of these constituents in normal rabbits as shown in table 1. The cholesterol and calcium levels are higher in the splenectomized than in the normal group,—cholesterol 134.3 in the splenectomized, 107.5 in the normal; calcium 14.9 in splenectomized, 12.5 in the normal. The blood sugar, total creatinine, preformed creatinine, uric acid, amino acid nitrogen, and inorganic phosphorus concentrations did not change to any appreciable extent as a result of splenectomy. The non-protein nitrogen and urea concentrations in the blood show some diminution in the splenectomized group.

Discussion. The chemical constituents in the blood are maintained with remarkable tenacity in normal conditions. Any agent causing impairment or deviation from the normal modus operandi in an animal results in significant alterations in the blood constituents. These are reflections of the metabolic activities taking place largely in the muscles. Any process which tends to increase the mass or accelerates the activity of active protoplasmic tissue invariably results in an increased inpouring of the products of metabolism into the blood stream, with the consequent elevation in the values of the chemical constituents.

The increases which we have noted in the nitrogenous constituents are entirely in harmony with the modern conception that such constituents play an important rôle in muscular contraction. Cannon's (1929) conception of an emergency endocrine that assists in the preparation of muscular concentration should be extended so that its action will include protein catabolism as well as carbohydrate. This fact was also emphasized by Watkins and Smith (1931) in their studies on the effect of adrenalin on nitrogen metabolism in rabbits.

It will be apparent from an inspection of table 1 that there is a definite parallelism between blood sugar and the nitrogenous constituents. There is a similar response of these substances to emotional stress. Sugar generally appears in the urine when blood urea rises; and puncture of the medulla (Dubois and Polonovski, 1924), results in an increase of urea in addition to glycosuria. Michaelis (1913) observed an increased excretion of uric acid when a diabetic puncture was made in experimental animals. He calls this the glycosuria (diabetic)-uric acid puncture. Tashiro (1925) found an increase in urea nitrogen in rabbits after binding.

Himwich and Fulton (1931) have demonstrated a lipemia in cats and dogs in emotional states. Since according to Cori and Cori (1928) adrenalin diminishes the oxidation of carbohydrates, therefore, despite the rise of the level of blood sugar, it does not seem that carbohydrate is the pre-

ferred foodstuff under violent emotional stress, but rather the increased concentration of blood fat is a result of the mobilization of fat depots in response to the call of the tissues for fuel. In view of the large increases in the products of protein catabolism in the blood stream, it appears clear that protein in addition to fat is used as foodstuff in the tissues in emotional stress.

Since cholesterol is closely associated with fat metabolism, we would expect a similar change in cholesterol. Our results indicate a cholesterolemia in emotionally excited rabbits. This cholesterolemia is not abolished but diminished by splenectomy—(fig. 1) +21.1 per cent in normal and +12.7 in splenectomized rabbits. The per cent increase in normal rabbits is practically twice that of the splenectomized group.

SUMMARY

1. This study was undertaken to determine the effect of emotional excitement on the changes in the blood chemical constituents in rabbits.

2. In emotional excitement the following blood constituents of normal rabbits were increased, viz.: glucose, non-protein nitrogen, urea, preformed creatinine, total creatinine, uric acid, cholesterol and hemoglobin. Inorganic phosphorus was decreased, but amino acids, calcium and chlorides showed practically no change.

3. In emotionally excited splenectomized rabbits changes in the blood constituents similar to those seen in normal rabbits occurred with the exception of hemoglobin which was little changed.

4. With our splenectomized rabbits in the quiet state the blood sugar level, total and preformed creatinine, uric acid, inorganic phosphorus, chlorides and hemoglobin were not markedly different from that found in the normal group in the same condition. The calcium and cholesterol levels were higher, while non-protein nitrogen and urea were lower than with the normal rabbits.

5. In the control experiments the first and second tests of all the blood constituents in the quiet state gave closely similar results.

6. This study indicates that proteins are used to supply energy in emotional states, as well as carbohydrates, and fats (Himwich and Fulton).

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THE EFFECT OF INITIAL TENSION AND LOAD ON THE RESPONSE OF THE NICTITATING MEMBRANE OF THE CAT

C. W. HAMPEL

From the Laboratories of Physiology in the Harvard Medical School

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The isometric response of skeletal muscle increases at first as the initial length of the muscle is increased, and after reaching a maximum rapidly decreases with further initial extension (Evans and Hill, 1914). The contractions of heart muscle at increased initial lengths provide a parallel phenomenon (Starling, 1918). That similar properties are displayed by smooth muscle has been shown for the intestine (Brocklehurst, 1926) and for the retractor penis (Winton, 1926). These studies on mammalian smooth muscle were complicated by the difficulties attendant upon isolation of the tissues and by the marked spontaneous activity present in these organs. Therefore, it was felt that additional information might be gained from an investigation on the nictitating membrane of the cat.

The nictitating membrane lends itself particularly well to the study of smooth muscle since registration of its contractions can be effected with the muscle in situ and with uninterrupted circulation, and its responses may be elicited either by stimulation of the cervical sympathetic nerve or by the introduction of adrenalin solutions into the circulation. Moreover, it ordinarily displays very slight tendency toward spontaneous contractions, and the limits of its physiological activity may be fairly well defined. The use, in this laboratory, of the smooth muscle of the nictitating membrane of the cat as an indicator of sympathetic activity (Rosenblueth and Cannon, 1932; Rosenblueth, 1932a, 1932b) has further suggested the advisability of determining to what extent initial extension affects the response of this smooth muscle.

METHOD. Anesthesia was effected by nembutal (Abbott). Ordinarily 0.7 cc. per kgm. intraperitoneally was sufficient to produce anesthesia deep enough to exclude eye movements which might cause passive shifts in the nictitating membrane (see Rosenblueth and Bard, 1932).

The head of the animal was firmly fastened in a head holder which was arranged to be lowered or raised by a screw clamp. Recording was effected by means of a thread passed through the cartilage in the nictitating membrane and directed in line with the external canthus of the eye, but slightly outward to avoid the curvature of the eyeball, to a tension lever. A lever

providing a 10-fold magnification and giving about 1 cm. excursion for 7 grams weight was found most suitable.

A maximal response was first obtained with the nictitating membrane slightly extended. Thereafter records were made, first with the thread so slack as to allow contraction without development of tension, and then with the head lowered an equal distance for each contraction until a series of isometric responses at increasing initial tensions and lengths was obtained. The procedure was then reversed, and responses recorded while the tissue was being released. An interval of 5 minutes to allow the tissue to assume its new length before stimulation was found to be sufficient; further change was very slight.

To determine the effect of load on the response of the nictitating membrane an isotonic lever with a 14-fold magnification was used. The points of membrane attachment and loading were on opposite sides of the fulcrum and at equal distances from it. Extension of the membrane due to loading was compensated for by lowering the head of the animal after each loading so that a constant base-line was maintained.

When excitation by intravenous injection of adrenalin solutions was used, the nictitating membrane was denervated acutely by removal of the superior cervical ganglion, and sensitized by cocaine (7.0 mgm. per kgm., intravenously). In addition, the adrenal glands were ligated. The adrenalin solution (1 cc., 1:50,000) was introduced during a 5-second interval through a cannula in the femoral vein.

For electrical stimulation of the cervical sympathetic nerve, maximal condenser discharges at a frequency of 15 per second from a helium tube circuit (similar to that used by Knoefel and Davis, 1933) were employed. The above frequency was chosen because it gave very nearly maximal responses (see Rosenblueth, 1932b) without affecting the magnitude of the response after many trials.

Results. The records of an experiment in which adrenalin injections were used for excitation of the nictitating membrane are shown in figure 1. Record A shows clearly the influence of increasing initial tension (and concomitant increase in initial length) upon the response of the smooth muscle, and record B depicts the same phenomenon at decreasing initial tensions and lengths.

The data obtained from the records in figure 1 are presented graphically in figure 2. The effect of increasing the initial tension upon the response is shown by the continuous curve; the effect of decreasing the initial tension is shown by the broken curve. The two curves are practically identical. The optimum initial tension, i.e., the initial tension at which the greatest response is obtained, is the same during extension and releasing phases. This fact is substantiated by results in other experiments, and what is more significant, the optimum initial tensions for observations on several mem-

branes show a remarkable constancy. The average optimum initial tension for 18 observations on 13 different preparations was 10.75 grams, with a probable error of ±0.37 gram. It may be noted that this constancy

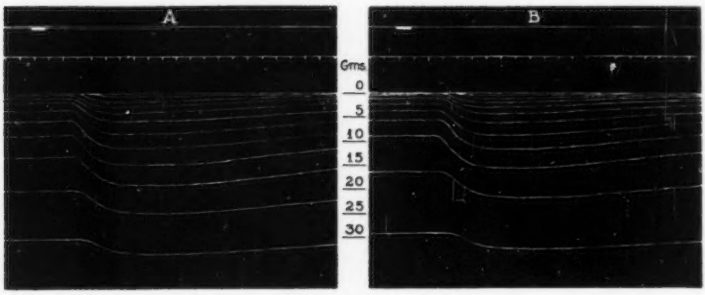

Fig. 1. Nembutal 0.7 cc. per kgm.; cocaine 7 mgm. per kgm. Adrenalin (1 cc. of 1:50,000) injected at signal. Time in 5-second intervals. Magnification, 10×. Calibration of tension lever in grams.

A. The nictitating membrane was extended 5 minutes before each response by lowering head 0.26 cm.

B. The nictitating membrane was released through the same stages.

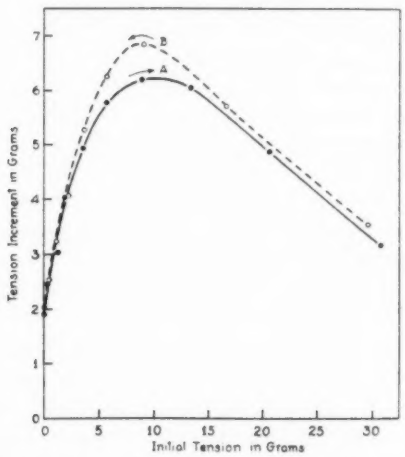

Fig. 2. From data in figure 1. Tension increment-initial tension curves, increasing initial tension, A; decreasing initial tension, B.

obtains whether responses are elicited through electrical stimulation of the cervical sympathetic nerve or through excitation by intravenous injections of adrenalin after cocaine sensitization.

Tension-length relationships for the nictitating membrane are also obtainable from the data in figure 1. The effect of stretching the membrane upon the tensions in the relaxed and contracted states is shown in figure 3a by the continuous curves *C* and *A* respectively. Similar tension-length relations while the tissue was being released through the same stages are shown by the broken curves *D* and *B*. It will be noted that the abscissa represents changes in length rather than absolute lengths of the membrane. This becomes necessary since the nature of the origin of the muscle renders accurate determination of absolute length impossible.

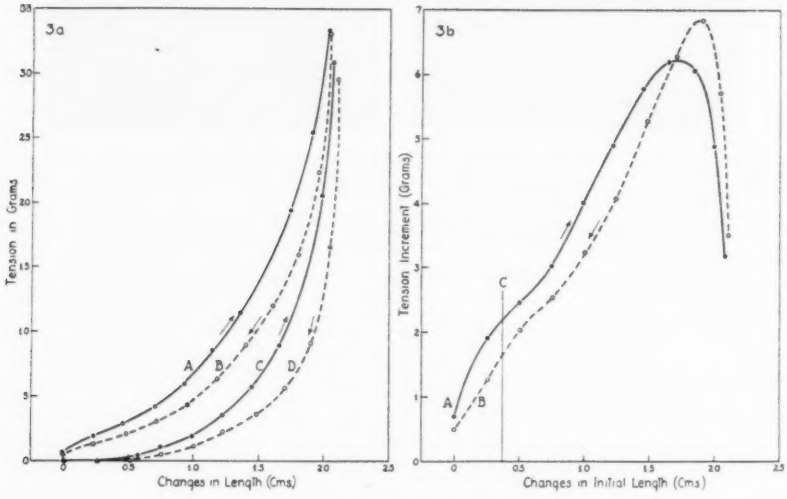

Fig. 3. From data in figure 1.

a. Tension-length curves for relaxed and contracted states, during stretching, *C* and *A*; during releasing, *D* and *B*.

b. Tension increment-length curves, during stretching, *A*; during releasing, *B*. Resting length shown by line *C*.

In this experiment the nictitating membrane was stretched nearly 2 cm. beyond its resting length. The greatest shortening upon contraction, however, was less than 1 mm. The differences between the tensions during the stretching and releasing processes call for some comment. That they are not due to any irreversible change of great extent in the smooth muscle of the membrane is evident from the approximation of the respective curves upon return to shorter initial lengths. The response after the tissue has assumed its resting initial length is very nearly as great as before being stretched. Curves for a similar procedure with the gut which are presented by Brocklehurst (*loc. cit.*) show greater differences between stretch-

ing and releasing tensions. He believed that the differences were due, in part at least, to the differences in elasticity of the tissues making up the gut. Since the nictitating membrane, like the intestine, contains a large proportion of non-contractile tissue (Zernik, 1928), the same reasoning may well apply to the data presented here.

The tension increment-length relationships for the same experiment are presented in figure 3b. The responses during the stretching phase are represented by the continuous curve; those during the releasing phase by the broken curve. The shapes of the two curves are very much the same.

The upward inflection in both curves at smaller initial lengths may well be due to minor mechanical difficulties in the recording apparatus and is of doubtful significance. The curves are quite similar to those obtained by Winton (*loc. cit.*) with the retractor penis. They are at variance, however, with those presented by Brocklehurst (*loc. cit.*) on the gut. The latter author found that the maximum tension-increment during the stretching phase occurred at a much smaller initial length than during the releasing phase. Brocklehurst makes no mention of any definite time interval between responses in his experiments, and it may therefore be of significance that in my experiments, as in Winton's, the changes in length were imposed very slowly. In the experiment reported above the membrane was stretched and released at a rate of less than 2 cm. per hour.

Experiments in which the nictitating membrane was made to respond by electrical stimulation of the cervical sympathetic nerve yield essentially the same results as those described above. The record of a typical experiment (fig. 4) and the tension increment-initial tension diagram (fig. 5) constructed from the data in the record are included for comparison. Such comparison brings out the fact that the cocaine used to sensitize the acutely denervated membrane when adrenalin excitation was used does not alter the tension increment-initial tension relationship. Experiments in which stimulation of the nerve was used after cocaine administration show only an augmented response (see Rosenblueth and Rioch, 1933) with no change in the tension increment-initial tension curves.

The influence of loading on the isotonic contraction of the nictitating membrane is presented in figure 6. The same base-line was maintained by lowering the head to compensate for the extension caused by each increment of loading. The height of contraction decreased progressively for each addition of load. The external work performed by the membrane (shortening $\times$ load) at different loads has been plotted in figure 7. Comparison of this curve with the curves in figures 2 and 5 reveals a striking similarity. Moreover, the value for optimum load (i.e., the load at which the greatest external work is done) in isotonic contraction and the value for optimum initial tension in isometric contraction are very nearly the same. As noted above, the average optimum initial tension was 10.75

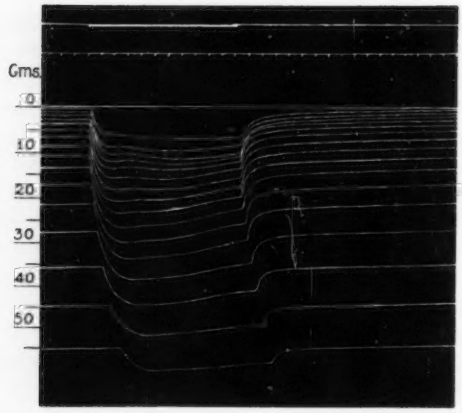

Fig. 4. Nembutal 0.7 cc. per kgm. Bipolar silver electrodes on the cervical sympathetic nerve. Stimulation (15 shocks per second) at signal. Time in 5-second intervals. Magnification, 8×. Calibration of tension lever in grams. The nictitating membrane was extended 5 minutes before each response by lowering head 0.19 cm.

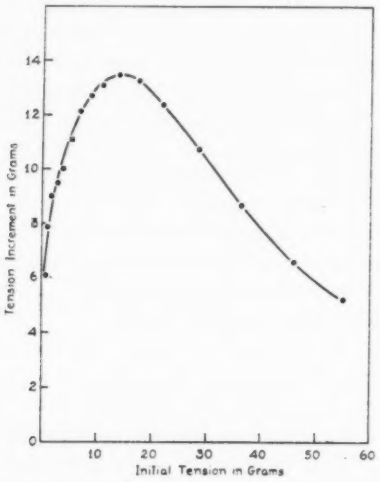

Fig. 5. From data in figure 4. Tension increment-initial tension curve during increasing initial tension.

± 0.37 grams. The average optimum load for seven different preparations was 11.57 ± 0.88 grams. It is reasonable to assume that the same amount of strain in the membrane is brought about by the same amount of

stress whether the stress be applied as a load or as a tension, and such a correlation between optimum load and optimum initial tension may well be taken to show that the maximum energy output occurs at approximately the same increase in initial length. Brocklehurst (*loc. cit.*) has shown that the optimum length-increase (i.e., the increase above resting length at which the greatest response is observed) for different pieces of ileum on stretching was extremely variable (from 4.0 to 52.6 per cent of the resting length). From the results on the nictitating membrane reported here the effects of increased initial tension and load (and the concomitant extension)

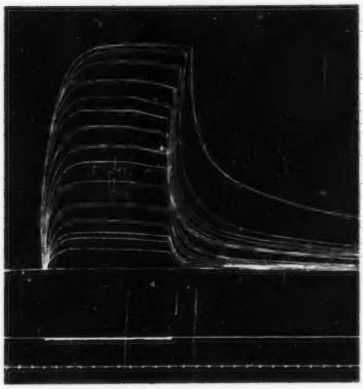

Fig. 6

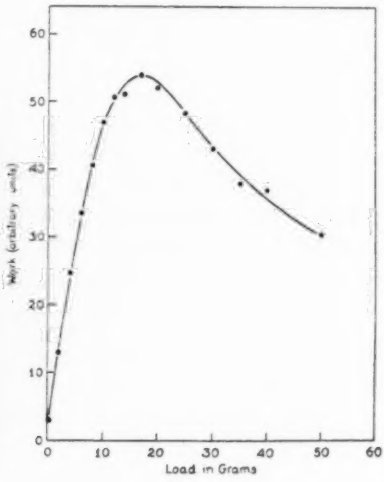

Fig. 7

Fig. 6. Nembutal 0.7 cc. per kgm. Bipolar silver electrodes on the cervical sympathetic nerve. Stimulation (15 shocks per second) at signal. Time in 5-second intervals. Isotonic lever; magnification, 14×. Effects of increasing load. Loads; 0.5 gram (lever), 2, 4, 6, 8, 10, 12, 14, 17, 20, 25, 30, 35, 40, 50 grams.

Fig. 7. From data in figure 6. Effects of increasing load on external work.

upon the mechanical energy liberated in contraction of smooth muscle appear to be more nearly predictable than Brocklehurst's work would indicate.

Attention may be directed, in passing, to the surprisingly great initial tensions and loads against which the nictitating membrane is capable of responding. Thus, in figure 4 the membrane displays an ability to contract against an initial tension of over 50 grams, and in figure 6 the membrane can be seen to have raised a load of 50 grams. The tension developed in isometric contraction may be as great as 13.5 grams.

It is of interest that the maximum response is reached only after the

nictitating membrane has been extended just beyond its greatest physiological length. In the animal used in figures 1, 2 and 3, the distance between the internal canthus and the external canthus of the eye was about 2.5 cm. Under conditions of deep anesthesia or following denervation, the membrane protrudes over the eye to a point about half-way toward the external canthus. This point is marked on figure 3b by line *C*. Reflex protrusion of the membrane in the normal animal (see Rosenblueth and Bard, *loc. cit.*) will bring the membrane well over the eyeball (about 1 cm. beyond *C*), and this latter position might well be taken as the extreme physiological extension of the nictitating membrane. The maximum response is not reached until the membrane has been extended beyond the external canthus. Skeletal muscle must also be extended beyond its greatest physiological length to obtain the maximum response (Beck, 1922; Davis and Davis, 1932).

SUMMARY

1. The effect of initial tension on the response of the smooth muscle of the nictitating membrane of the cat has been investigated. Tension increment-initial tension relations are affected similarly by increasing or decreasing the initial tension (fig. 2). The average optimum initial tension for 18 observations on 13 nictitating membranes was found to be 10.75 ± 0.37 grams. The tension increment-initial tension relations are the same whether excitation is effected through intravenously injected adrenalin or through stimulation of the cervical sympathetic nerve (figs. 2 and 5).

2. Tension-length curves for relaxed and contracted states of the nictitating membrane during stretching and releasing stages are presented (fig. 3a). The tension increment-length curves during stretching and releasing stages are practically identical (fig. 3b).

3. The influence of load on the isotonic response of the smooth muscle of the nictitating membrane has been determined (fig. 6), and the effect of increasing load on the external work is graphically presented (fig. 7). The average optimum load for 7 different preparations was found to be 11.57 ± 0.88 grams.

4. The maximum response of the nictitating membrane in isometric contraction is reached only after extension has been carried beyond the greatest physiological length.

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